ANTIMICROBIAL USAGE IN COMPANION AND FOOD ANIMALS: METHODS, SURVEYS AND RELATIONSHIPS WITH ANTIMICROBIAL RESISTANCE IN ANIMALS AND HUMANS

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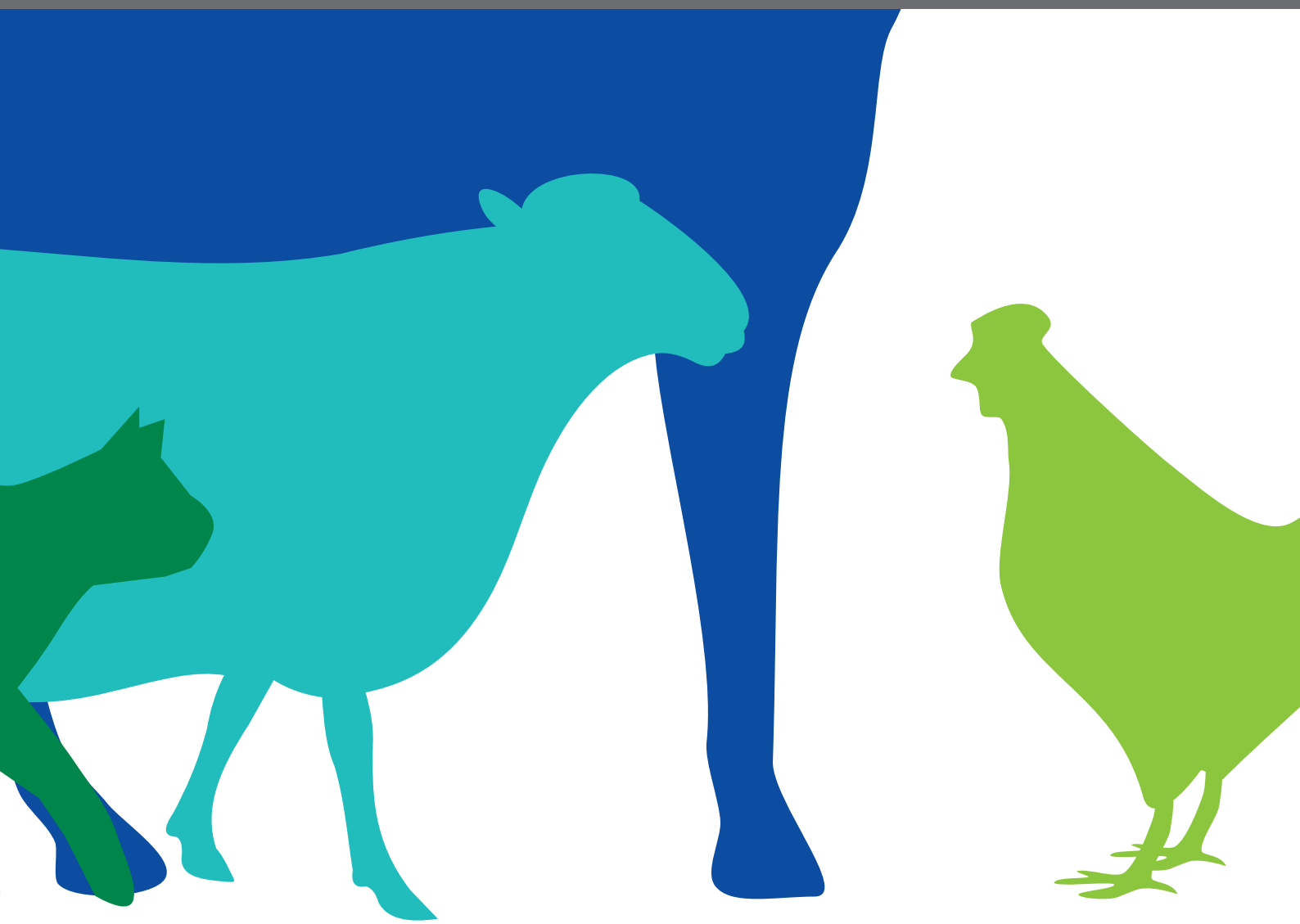

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ANTIMICROBIAL USAGE IN COMPANION AND FOOD ANIMALS: METHODS, SURVEYS AND RELATIONSHIPS WITH ANTIMICROBIAL RESISTANCE IN ANIMALS AND HUMANS

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EDITORIAL
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Editorial: Antimicrobial Usage in Companion and Food Animals: Methods, Surveys and Relationships With Antimicrobial Resistance in Animals and Humans

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Editorial on the Research Topic

Antimicrobial Usage in Companion and Food Animals: Methods, Surveys and Relationships With Antimicrobial Resistance in Animals and Humans

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The best way to quantify antimicrobial use (AMU) in animals is still an elusive question, it probably does not have a unique answer. This collection of 15 articles describes different metrics, methodologies, data sources, animal scenarios, study designs, and levels of study about AMU quantification in animals. The diversity of approaches highlights a strong need for international collaboration, sharing of experiences, and more discussion about methods to improve uptake of harmonized standards (where harmonization might be suitable).

The less controversial aspect of this topic is that there was consensus among these articles that a relative measure is needed, dividing amounts of antimicrobials (numerator) by a denominator summarizing the animal population at risk of being treated. However, both the numerator and denominator have their specific challenges. In addition, a period of time for data collection must be fixed or considered. In the human arena, a standardized population approach based on established defined daily doses (DDDs) and census data is utilized around the world, delivering information on the number of DDDs per population and year (or days) (1). Nevertheless, mimicking this procedure in animals is not an easy subject, with many controversial facets previously described (2).

The use of weight-based (e.g., mg or kg of active ingredient) vs. dose-based (e.g., DDD) metrics in the numerator was discussed in several papers of this collection. A main advantage of weight-based metrics is their higher availability (i.e., the data comprising these metrics are more often available), that make them a more accessible option for worldwide AMU monitoring (Góchez et al.). However, Brault et al. demonstrated that dose-based metrics were more accurate than weight-based metrics when there was variation in the type (e.g., concentrations and durations of effect) of antimicrobials used by the populations being compared. The studies by Agunos et al., Brault et al., and Van Cuong et al., where weight-based and dose-based metrics were applied to the same AMU data, all demonstrated a significant impact of those metrics on the study results, that could even lead to different conclusions (e.g., increase vs. decrease in AMU over time in turkeys in Agunos et al.). Agunos et al. stressed the added value of using multiple AMU indicators for monitoring the impact of stewardship activities and interventions. Nonetheless, weight-based and dose-based metrics are not mutually exclusive, and it is possible to convert one into another (Stebler et al.).

Two articles in this collection addressed defining or establishing national "animal" or "vet" (a linguistic discussion not yet resolved) DDDs for pigs (Bosman et al. in Canada; Echtermann et al. in Switzerland) and poultry (Bosman et al. in Canada), as a tool for the calculation of the number of DDDs per animal population and year (a proxy of the number of treatments-day), at the country or region-level. This indicator is also discussed and used in the article of Brault et al. In addition, national vet defined course doses (DCDs) for pigs have been proposed in Switzerland in the article of Echtermann et al. to calculate the number of DCDs per population and year (a proxy of the number of treated animals). A similar exercise for calculation of the number of treated animals in pigs and calves in Switzerland is presented in the article of Stebler et al. National DDDs lists proposed by Bosman et al. and DDDs and DCDs lists proposed by Echtermann et al. differed from those proposed by the European Medicines Agency for certain antimicrobial classes (3), reflecting the need for individual countries to develop their own lists for more precise AMU quantification at the national level, while the EMA lists may be preferred for international comparisons.

Both the number of DDDs and the number of DCDs are indicators based on standardized measurements that do not necessarily reflect the real or actual AMU. For a more real AMU estimation in a given population with detailed data available, the used daily dose (UDD) may be a better choice to reflect what is happening in that specific population in terms of selection pressure. This is explored in the articles of Kasabova et al. (pigs and broilers), Brault et al. (beef feedlot), and Waret-Szkuta et al. (pigs). All three papers highlighted that the choice of DDD vs. UDD had an impact on the results. Interestingly, Kasabova et al. recommended using UDD-based calculations to run monitoring systems with a benchmark mission. Should DDD be preferred to compare AMU between populations, additional considerations should be made to adjust for discrepancies between DDD and UDD.

The third parameter having a huge effect on AMU indicators is the animal weight. The article of Brault et al. addressed this question in beef cattle, where the use of estimated vs. actual weights notably influenced the results obtained. Similar observations were made in pigs by Waret-Szkuta et al. Equally, the use of weight at treatment vs. the weight at slaughter (Góchez et al.) or the weight sold (Van Cuong et al.) had a strong impact on calculations, especially for larger livestock species like cattle and pig.

Sales of veterinary medical products containing antimicrobials are a classical source of raw data for AMU consumption calculations (Góchez et al.; Stebler et al.). Nevertheless, prescriptions because they have more detailed information closer to the end-users, may be a more accurate source of possible selection pressure; prescriptions were used in the articles of Hommerich et al. and Hopman et al. to calculate AMU in German cattle and Dutch companion animals, respectively.

Although most of the literature on AMU in this collection focused on pigs and cattle (half of the articles of this Research Topic), four articles considered AMU in poultry (Kasabova et al.; Van Cuong et al.; Agunos et al.; Bosman et al.), all of them using the above mentioned DDD approach. Data on AMU from pets were presented in three articles (Singleton et al.; Gómez-Poveda and Moreno; Hopman et al.), using different approaches. Hopman et al. used DDD per clinic and year, whereas Singleton et al. and Gómez-Poveda and Moreno focused on the percentages of prescriptions. Specific scenarios regarding indications for AMU, such as bovine respiratory disease (Brault et al.) and canine acute diarrhea (Singleton et al.) were also presented in this collection, as well as an article exploring drivers for AMU in the pig sector presented by Coyne et al..

Finally, the OIE approach for worldwide AMU monitoring was described in the article of Góchez et al. The OIE view and efforts on this topic are of paramount

TABLE 1 | This table includes a list of options noted in the research collection for consideration based on available data or objectives of AMU reporting.

	Basic data	**Examples**
AMU (numerator)	Data source	Sales, prescriptions, invoices, farm records, others
	Level of measurement	Individual animal, batch/flock/pen, farm, region, country, others
	Timing coverage	Year, production cycle, others
	Dose	Standard (SPC), used, others
	Treatment length	Standard (SPC), used, others
	Index	DDD, UDD, DCD, UCD, mg, kg, others
Population (denominator)	Data source	Farm records, national data bases, census information, FAOSTAT, others
	Level of measurement	Individual animal, batch/flock/pen, farm, region, country, others
	Timing coverage	Year, production cycle, others
	Body weight level	At treatment, at slaughter, at sale, others
	Body weight	Standard (e.g., average weight at treatment), measured, others
	Index	Biomass, population correction unit, number of animals, number of animal-time, others
Indicator	Denomination	Mg of active substance/biomass, Number of DDDs per (10x) animal-time at risk, number of UCD per (10x) animal at risk, others

SPC, summary of product characteristics; DDD, defined daily dose; UDD, used daily dose; DCD, defined course dose; UCD, used course dose.

importance for understanding the different situations around the world where the data may be obtained, and the compromise for a global harmonized methodology to report quantitative data.

In summary, several articles of this collection highlighted that real use data (regarding dose, treatment length and body weight at treatment) were the ideal data for calculating and reporting AMU. Nevertheless, all these data are rarely available simultaneously, hence standard values are the pragmatic alternate choice for calculations. Consequently, transparency about the methods and data used to calculate AMU indicators is needed (**Table 1**). This was stressed by all the authors in this collection as a pre-requisite to preserve accuracy and understanding of the data, especially when data comparisons are performed.

AUTHOR CONTRIBUTIONS

MM produced the first draft of the editorial. All authors edited and approved the editorial.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer JD declared a past co-authorship with one of the author LC to the handling editor.

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ORIGINAL RESEARCH
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Antimicrobial Prescriptions for Dogs in the Capital of Spain

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Objective: To characterize antimicrobial prescription patterns for dogs in veterinary practices in Spain using the city of Madrid as a model.

Design: Retrospective survey.

Settings: Dogs attending veterinary practices in the city of Madrid in 2017 were enrolled.

Subjects: Three hundred dogs from 30 veterinary practices randomly selected from a set of 388 practices grouped by zip code. The inclusion criterion for dogs was treatment with antibiotics within a few days of the data collection day.

Results: For the 300 dogs enrolled, 374 treatments with antimicrobials were recorded, 62.8% (235/374) were veterinary medicinal products and 37.2% (139/374) human medicinal products. The main route of administration was oral (209/374; 55.9%) followed by parenteral (100/374; 26.7%) and topical (65/374; 17.4%). Sixty-five dogs (21.7%) received a perioperative antimicrobial treatment, mainly associated with female obstetrical surgery (19/65; 29%), while 78.3% (235/300) received a pharmaceutical treatment mainly for skin (72/235; 30.6%), respiratory (47/235; 20%), or digestive (41/235; 17.4%) diseases. The most frequently used antimicrobials were beta-lactams for oral (119/209) and parenteral (79/100) administration, especially the combination amoxicillin with clavulanic acid (83/209; oral), amoxicillin alone (42/100; parenteral), and aminoglycosides (32/65) for topical use. Diagnostic confirmation with culture was carried out on only 13 out of 235 dogs receiving therapeutic treatment and nine underwent an antimicrobial susceptibility test. In addition, cytology was performed in 15 dogs.

Conclusions: The pattern of antimicrobial prescriptions for dogs in our study was quite similar to that previously described in several European countries, and encompassed the same two highly interconnected key features: major use of amoxicillin with clavulanic acid and a very low level of antimicrobial susceptibility testing before prescription. Consequently, we recommend that the measures for rationalizing antimicrobial prescription for dogs in Spain should follow those implemented in other countries, especially confirming the diagnosis and promoting the use of hygiene measures by owners.

Keywords: antibiotics, pets, survey, beta lactams, prescriptions, conditions

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INTRODUCTION

Antimicrobial resistance is currently one of the leading public health risks and antimicrobial usage one of its key drivers, both in humans and animals.

Antimicrobial resistance in animal bacteria (zoonotic, pathogenic for animals or commensal) is of great concern, especially if resistant bacteria can be spread to humans. Foodborne transmission is the most frequently studied route, but some authors have raised awareness about the increasing importance of direct contact transmission with pets (1, 2) (for veterinary surgeons and owners, especially children) and food animals (for workers, veterinary surgeons, etc.).

Antimicrobials (AM) are frequently prescribed for companion animals in the treatment of various conditions. Due to high public health concern, there are an increasing number of guidelines for prudent or responsible use of antimicrobials (see for example World Health Organization (http://www.who.int/foodsafety/publications/cia_guidelines/en/); Federation of Veterinarians of Europe (https://www.fecava.org/sites/default/files/files/fve_antimicrobials_pets_final_small.pdf) or Responsible Use of Medicines in Agriculture Alliance (https://www.ruma.org.uk/antimicrobials/guidelines/). Although factors influencing antibiotic prescribing habits of veterinary surgeons are not universal (3), those for veterinary surgeons of companion and food animals are quite similar. They include self-training, literature reviews, official reports, and commercial information (3).

Some human medicinal products (MP) containing antimicrobials are also used for companion animals (2), according to the prescribing "cascade" procedure (4) (articles 10 and 11 of Directive 2001/82/EC). Uses deviating from the Summary of Product Characteristics (SPC) are called off-label use (5). Current regulation of veterinary medicinal products (VMP) in the EU allows veterinary surgeons (under certain circumstances, to avoid causing unacceptable suffering to diseased animals, and under their own responsibility) to prescribe human MP for animals. A reflection paper on off-label use has been publishe by the European Medicines Agency (EMA) (5).

In the European Union (EU), sales of VMP containing antimicrobials have been compiled by the EMA from data provided by national authorities since 2010. This was in response to a 2008 mandate from the EU Commission (6). The EMA publish a yearly European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) report on antimicrobial sales that mostly covers food animals and produces a national indicator relating antimicrobial sales and animal biomass expressed as milligram of antimicrobials per population correction unit. This is an overall indicator covering the major food animal species but is not specific for any species. Although the authorized data sheets for dog products, typically in the form of tablets but also injectable, are provided by some participating countries, the Statistical Office of the European Union (Eurostat) does not have accurate data on dog and cat populations, and consequently, they are not included in the national indicator mentioned above. According to the last ESVAC report (6), sales of tablets accounted for <8% of total antimicrobial sales in all the countries, except Iceland, Finland, Norway, and Sweden.

Total amounts of antimicrobials (sales or consumption data) are not the only approach for understanding the selective pressure for antimicrobial resistant bacteria. Complementary information such as patterns of use of antimicrobials according to animal species, conditions, etc. is also of value. Some information about these patterns in dogs already exists, especially from the UK (7–9), but also from Finland (10), Italy (11), and Australia (12). There are no current data available for Spain although, according to the 2016 ESVAC report (6), Spain was ranked as the second EU country by antimicrobial sales in animals.

The aim of this survey was to characterize antimicrobial prescriptions in dogs in a random sample of veterinary practices in the city of Madrid, Spain, assuming that they could be used as a rationale estimate of prescriptions throughout the country.

MATERIAL AND METHODS

Sampling Frame

A sampling frame of 388 veterinary practices treating dogs in the city of Madrid (comprising about 1,000 practitioners) was constituted in December 2016 with data from the websites of the Official Veterinary Professional Association of Madrid (https://www.colvema.org/sac_lis_clinicas.asp) and a phone book web page (www.paginasamarillas.es). Veterinary practices were grouped by zip code, obtaining 52 zip codes that had at least one veterinary practice (from 1 to 14 veterinary practices per zip code).

Sampling Design

From these 52 zip codes, 30 were randomly selected and then one veterinary practice was also randomly selected from each zip code. Finally, 10 dogs attending the 30 veterinary practices who agreed to collaborate with the survey were included in the study on the basis of having recently received an antimicrobial prescription prior to being contacted during 2017.

Data Collection

Veterinary surgeons in charge of the enrolled veterinary practices were contacted by phone to confirm their willingness to participate in the survey. After verbal agreement, a physical meeting at their facilities was convened for compiling information from their records of case histories.

Data Collection Form

The data collection form (in Spanish and available as **Supplementary Material**) contained questions regarding the dog (sex, breed, birth date, and weight), current condition (date, clinical signs, diagnostic, bacteriological culture, antimicrobial susceptibility test, and other diagnostic tests) and the antimicrobial prescriptions or administration in the practice (commercial name, active substance, administration route, posology, pharmaceutical form, and prescription type), considering all antimicrobials prescribed on the same record. Amoxicillin plus clavulanic acid was considered a single drug

for the purposes of data collection. Any personal information regarding the pets' owners was also recorded.

Data Recording and Analysis

Data were recorded in Microsoft Excel and a descriptive analysis performed with the same program and with the IBM SPSS Statistics software, version 22.

Conditions (Treatment Indication)

We distinguished two main uses of MP. When a dog received a MP for a condition, this was classified as a therapeutic treatment. Whereas when a dog received the MP as part of a surgical procedure (administered prior, during or after the surgical procedure), the administration was classified as prophylactic. In addition, we grouped therapeutic treatments according to the main systems/organs involved (skin, mouth, digestive tract, respiratory tract, ear, eye, urinary, and other), and prophylactic treatments according to similar medical criteria (obstetrics, male genitourinary operation, odontology, traumatology, dermatology, and other).

Antimicrobial Prescription Assessment

The recorded use of all VMP was checked against their respective Summary of Product Characteristics (https://cimavet.aemps.es/cimavet/medicamentos.do) for compliance with target species, indications for use (condition), and posology (dosage and duration).

RESULTS

Sample Description

Overall during the study, we contacted 50 veterinary practices to recruit the 30 practices who eventually agreed to participate in this survey. The 30 practices included in the study belonged to 29 of the 30 zip codes randomly selected in the original sampling. Because none of the veterinary practices belonging to one zip code was able to participate, this zip code was replaced by another on the list. In summary, 18 of the 30 veterinary practices originally selected for the study were willing to participate, whereas 12 were replaced.

Of the 300 dogs participating in the survey, 174 (58%) were male and 42% (126/300) female. Their age ranged from three months to 17 years (mean = 5.9 years; standard deviation = 4.5). The dogs were classified into 49 breeds, with 93 of the dogs (30.7%) being crossbreeds.

Diagnostic Tests for Bacterial Infection

Bacteriological culture had been performed for 5.5% (13/235) of the dogs receiving a therapeutic treatment (six ear, three urinary, two skin, and two digestive conditions) and an antimicrobial susceptibility test for 3.8% of the dogs (9/235) (five ear, three urinary, and one skin conditions). Cytology testing was performed in 6.4% (15/235) cases (five ear, four skin, one urinary, and five miscellaneous conditions).

Medicinal Products

We documented 374 MP containing antimicrobials from the medical records of 300 dogs (**Table 1**), prescribed between January and July 2017. Two hundred and thirty-two dogs received one product, 63 dogs received two products, four dogs received three products and one dog four products.

Based on the data sheets, 62.8% (235/374) of the products were for veterinary use and 37.2% (139/374) were for human use (including 15 extemporaneously prepared products; **Table 1**).

The most common administration route was oral (209/374; 55.9%; **Table 2**), followed by parenteral (100/ 374; 26.7%; **Table 3**), and topical (65/374; 17.4%; **Table 4**).

Antimicrobials

Of the 374 products, 93.6% (350/374) contained one single antimicrobial, while the remaining 6.4% (24/374) combined two (metronidazole - spiramycin; sulfadoxine - trimethoprim; benzylpenicillin - dihydrostreptomycin and polymyxin B - neomycin) or three (formosulfathiazol—dihydrostreptomycin - neomycin).

The 374 products contained 26 different antimicrobials (**Table 1**), with beta-lactams (201/374; 53.7%) being the most widely used antibiotic class by far, followed by fluoroquinolones (46/374; 12.3%), aminoglycosides (41/374; 11%), and imidazole derivatives (36/374; 9.6%). Of the active ingredients, amoxicillin with clavulanic acid was the most common, followed by amoxicillin, cephalexin, and metronidazole. Four of the identified antimicrobials (ciprofloxacin, tobramycin, azithromycin, and mupirocin) were not authorized for veterinary use in Spain.

The distribution of antimicrobial treatments according to the administration route showed that most of them fell into systemic (oral or parenteral) or topical (skin, eye, ear) use. Beta-lactams, macrolides, lincosamides, tetracyclines, sulphonamides, trimethoprim, and metronidazole were always used systemically (**Tables 2**, **3**), whereas polymyxins, phenicols, fusidic acid, and mupirocin were only used topically (**Table 4**). Fluoroquinolones were mainly for systemic use but some topical products contained marbofloxacin. Aminoglycosides were mostly used topically, although streptomycin and neomycin were sporadically employed systemically.

Conditions (Treatment Indication)

Two hundred and thirty-five out of 300 dogs (78.3%) received a therapeutic treatment with an antimicrobial product, whereas 65 out of 300 dogs (21.7%) received a prophylactic (perioperative) treatment (35 after surgery, 21 during the intervention and nine prior to surgery). Surgical procedures included the following interventions: obstetrical (19 of 65 dogs; 29%), male genitourinary (14/65; 22%), dental (8/65; 12%), skin (8/65; 12%), traumatological (6/65; 9%), and other (10/65; 15%; **Table 5**).

The most common general conditions for therapeutic use of antimicrobials were skin disorders (72/235; 30.6%), respiratory disorders (47/235; 20%), and digestive disorders (41/235; 17.4%). The specific diseases that were more frequent in skin were dermatitis (20/235; 8.5%) and pyoderma (9/235;3.8%); in the respiratory tract, kennel cough (39/235; 16.6%); in the digestive system, enteritis (20/235; 8.5%) and gastroenteritis (14/235; 6%); in the ear, external otitis (30/235; 12.8%); in the eye, conjunctivitis (12/235; 5.1%), and in the urinary tract, cystitis (15/235; 6.4%).

TABLE 1 | Distribution of antimicrobials of 374 medicinal products (MP) prescribed to 300 dogs (Madrid City) according to authorization for Veterinary (VMP) or Human (HMP) use.

Antimicrobials	VMP	HMP	Total MP	% over 374 MP	Dogs	% over 300 dogs
Beta-lactams	**146**	**55**	**201**	**53.7**	**187**	**62.3**
Amoxicillin-clavulanic acid	61	42	103	27.5	91	30.3
Amoxicillin/ampicillin	43	1	44	11.8	44	14.7
Benzylpenicillin**	2		2	0.5	2	0.7
Cefalexin	29	12	41	11.0	39	13.0
Cefovecin	10		10	2.7	10	3.3
Cefquinome	1		1	0.3	1	0.3
Fluoroquinolones	**41**	**5**	**46**	**12.3**	**44**	**14.7**
Marbofloxacin	23		23	6.1	23	7.7
Enrofloxacin	18	1	19	5.1	17	5.7
Ciprofloxacin		4	4	1.1	4	1.3
Aminoglycosides	**8**	**33**	**41**	**11.0**	**41**	**13.7**
Neomycin	6	13	19	5.1	19	6.3
Tobramycin		12	12	3.2	12	4.0
Gentamicin		5	5	1.3	5	1.7
Dihydrostreptomycin	2	3	5	1.3	5	1.7
Imidazole derivatives	**8**	**28**	**36**	**9.6**	**36**	**12.0**
Metronidazole	8	28	36	9.6	36	12.0
Polymyxins	**12**	**3**	**15**	**4.0**	**15**	**5.0**
Polymyxin B	12	3	15	4.0	15	5.0
Tetracyclines	**8**	**6**	**14**	**3.7**	**14**	**4.7**
Doxycycline	8	6	14	3.7	14	4.7
Macrolides and lincosamides	**7**	**8**	**15**	**4.0**	**15**	**5.0**
Spiramycin***	6	3	9	2.4	9	3.0
Clindamycin		3	3	0.8	3	1.0
Azithromycin		2	2	0.5	2	0.7
Tylosin	1		1	0.3	1	0.3
Sulphonamides	**3**	**8**	**11**	**2.9**	**11**	**3.7**
Sulfadoxine -trimethoprim	3	5	8	2.1	8	2.7
Formosulfathiazol		3	3	0.8	3	1.0
Others	**10**	**2**	**12**	**3.2**	**12**	**4.0**
Florfenicol	6		6	1.6	6	2.0
Fusidic acid	4		4	1.1	4	1.3
Mupirocin		2	2	0.5	2	0.7

*Combinations: Metronidazole-spiramycin; sulfadoxine-trimethoprim; Benzylpenicillin-dihydrostreptomycin; polymyxin B-neomycin; formosulfathiazol-dihydrostreptomycin-neomycin; **Always in combination with streptomycin; ***Always in combination with metronidazole.*

Assessment of Prescription Compliance With the Summary of Product Characteristics (SPC)

The data recorded from the practitioners when using the 235 VMP were checked against the SPC for compliance with target species, indications for use (condition) and posology (dosage and duration; **Table 6**). Only 15 VMP did not list dogs as the target species (all of these were authorized for several other food animals). The condition treated was listed in the indication for use in 64.3% of the products, with the lowest compliance recorded for digestive disorders. Compliance with recommended dosage fluctuated between 42 and 94%, with overdosage (23%) more common than underdosage (12.8%). In summary, 40.4% of the VMP were used in accordance with the SPC. The use in dogs of human products was not evaluated.

DISCUSSION

Survey Design and Potential Biases

Veterinary teaching hospital records (10, 11) and veterinary practice electronic records in private databases (7–9) were used as sources of data in previous studies, none of which could be considered as census studies at their respective national level. Nevertheless, all of them were able to draw reasonable pictures of antimicrobial prescription in dogs that could be generalized to their countries. This survey was based on a random selection

TABLE 2 | Distribution of antimicrobials of 209 oral medicinal products according to organ/system (conditions) treated, on 300 urban dogs (Madrid City).

	Respiratory	Urinary	Skin			Eye	Ear	Digestive		Surgical	Others
Antimicrobial class			**Dermatitis**	**Bite**	**Folliculitis**	**Corneal Ulcers**	**Otitis**	**Gastroenteritis**	**Gingivitis**		
Single (194)	35	14	21	9	3	2	10	25	5	31	39
Combinations (15)		1		1				5	2	3	3
Beta-lactams	**20**	**7**	**17**	**6**	**3**		**5**	**2**	**5**	**20**	**28**
Amoxicillin-clavulanic acid	20	7	5	3			3	2	5	20	17
Amoxicillin/ampicillin			1								1
Cephalexin			11	3	3		2			7	10
Fluoroquinolones	**4**	**6**	**3**	**2**		**1**	**5**	**1**		**1**	**7**
Marbofloxacin	1		3	2		1	3				6
Enrofloxacin	3	4					1	1			1
Ciprofloxacin		2					1			1	
Nitroimidazoles				**1**				**22**			**1**
Metronidazole				1				22		1	1
Tetracyclines	**9**	**1**				**1**				**1**	**2**
Doxycycline	9	1				1				1	2
Macrolides and lincosamides	**2**		**1**							**1**	**1**
Clindamycin			1							1	1
Azithromycin	2										
Combinations											
Sulfadoxine-trimethoprim		1						1			1
Metronidazole-spiramycin				1				1	2	3	2
Formosulfathiazol-dihydrostreptomycin-neomycin								3			

of 30 veterinary practices located in the city of Madrid but relied on the willingness of the practitioners to participate. This could have biased the sample in favor of those more likely to collaborate with the Veterinary Faculty or those specifically interested in the topic. Nonetheless, 60% (18/30) of the effective participants belonged to the random sample selected, whereas all but one of the remainder came from randomly selected zip codes. This gives some confidence that the sample was representative.

Most of the treatments were prescribed in winter and spring (from January to April 2017), which could have produced a seasonal bias in favor of the conditions that are more common during this period.

Finally, the information recorded on the case history of the dogs was quite diverse and many difficulties arose when we tried to cluster treatments based on clear indications, as mentioned in the materials and methods section. This might produce discrepancies when antimicrobials per condition are compared to other studies.

Antimicrobials

Antimicrobial preparations are most frequently administered by the oral route in dogs (8, 10, 11) and our survey confirmed this finding. Most of the authors studying antimicrobials in dogs (7–9, 11, 12) reached the conclusion that amoxicillin with clavulanic acid was by far the most frequently used systemic antimicrobial and our survey showed the same result. Nevertheless, there are few clinical reasons that support such extensive use. The Danish antibiotic use guidelines for companion animal practice (13) only classified amoxicillin with clavulanic acid as a first option antibiotic for a short list of bacterial infections (pneumonia, furunculosis, otitis media, pyelonephritis, acute metritis, orchitis/epididymitis, and dacryocystitis) most of them infrequent in dogs. Guardabassi et al. (14) also compiled a similar list including pneumonia, central nervous system infections, pyelonephritis, and pyoderma produced by isolates of *Staphylococcus pseudintermedius* susceptible to amoxicillin with clavulanic acid, which is the only condition where amoxicillin with clavulanic acid is the first option in the Swedish guidelines (15) for the clinical use of antibiotics in the treatment of dogs and cats.

As in our survey, amoxicillin and cephalexin were among the most common systemically used antimicrobials after amoxicillin with clavulanic acid (7–11). Cephalexin was reported to be the most commonly prescribed drug for pyodermas (10, 16), traumatic wounds and surgical procedures (10). Consequently, the beta-lactams class (penicillins and cephalosporins) were at the top of the prescription list, both in the overall rank of systemic antimicrobials and for several specific conditions affecting the

TABLE 3 | Distribution of antimicrobials of 100 parenteral medicinal products according to organ/system (conditions) treated, on 300 urban dogs (Madrid City).

	Respiratory	Urinary	Skin		Ear	Digestive	Surgical	Others
Antimicrobial class			**Dermatitis**	**Bite**	**External Otitis**	**Gastroenteritis**		
Single (93)	19	4	6	4	1	11	31	17
Combinations (7)	1					6		
Beta-lactams	**17**	**3**	**5**	**4**	**1**	**6**	**29**	**14**
Amoxicillin-clavulanic acid	7			1		3	9	1
Amoxicillin/ampicillin	6	2	2	2	1	3	17	9
Cephalexin	3			1				1
Cefovecin	1	1	3				2	3
Cefquinome							1	
Fluoroquinolones	**2**	**1**	**1**			**2**	**2**	**3**
Marbofloxacin	1						1	
Enrofloxacin	1	1	1			2	1	3
Ciprofloxacin								
Nitroimidazoles						**2**		
Metronidazole						2		
Macrolides and lincosamides						**1**		
Tylosin						1		
Combinations								
Benzylpenicillin-dihydrostreptomycin	1					1		
Sulfadoxine-trimethoprim						5		

TABLE 4 | Distribution of antimicrobials of 65 topical medicinal products according to administration route and conditions treated, on 300 urban dogs (Madrid City).

	Ocular			Otic	Cutaneous		
Antimicrobial class	**Conjunctivitis**	**Corneal ulcers**	**Others**	**Otitis**	**Dermatitis**	**Ulcers**	**Others**
Single (63)	13	5	4	24	9	3	5
Combinations (2)		1		1			
Fluoroquinolones				**5**			
Marbofloxacin				5			
Aminoglycosides	**13**	**5**	**2**		**6**	**2**	**5**
Neomycin	2	1	1		5	2	5
Tobramycin	7	4	1				
Gentamicin	4				1		
Polymixyns			**2**	**11**			
Polymyxin B			2	11			
Others							
Florfenicol				6			
Fusidic acid				2	2		
Mupirocin					1	1	
Combinations							
Polymyxin B-neomycin		1		1			

skin (10, 11, 17), gastrointestinal tract, eyes, respiratory system, musculoskeletal system (10, 11), genitourinary, and respiratory systems (17).

Ranked from most to least commonly prescribed, the antimicrobial classes following beta-lactams for systemic use differ between countries and show different patterns. In the UK (7, 8), the next most common were nitroimidazoles, lincosamides and macrolides, and fluoroquinolones. In the Nordic countries [Sweden and Norway (18); Finland (10)], amoxicillin with clavulanic acid was followed by trimethoprim-sulphonamides, macrolides and lincosamides, fluoroquinolones, and metronidazole. In an Italian study with dogs and cats

TABLE 5 | Distribution of antimicrobials of 65 medicinal products for perioperative use according to type of surgery.

Surgery	Amoxicillin with clavulanic acid	Amoxicillin	Cephalosporins	Fluoroquinolones	Others
Obstetrics (19)	11	4	2 (cephalexin) 1 (cefovecin)		1 (metronidazole)
Genito-urinary male (14)	7	5	2 (cephalexin)		
Dental (8)	1	2	1 (cephalexin) 1 (cefovecin)		2 (metronidazole-spiramycin) 1 (clindamycin)
Skin (8)	4	2		1 (enrofloxacin)	1 (doxycycline)
Traumatology (6)	2	1	2 (cephalexin)	1 (marbofloxacin)	
Others (10)	4	3	1 (cefquinome)	1 (ciprofloxacin)	1 (metronidazole-spiramycin)

TABLE 6 | Assessment of compliance with the summary of the product characteristics (SPC) of 235 veterinary medicinal products (VMP) prescribed to 300 dogs.

Condition	Target species	Condition	Dosage	Duration	All	Over dosage	Under dosage
Skin (61)	98%	80%	70%	80%	49%	23%	7%
Digestive (24)	71%	29%	42%	71%	13%	25%	13%
Respiratory (40)	95%	60%	53%	78%	35%	18%	28%
Ear (31)	100%	90%	94%	97%	81%	3%	3%
Eye (2)	2/2*	0/2	1/2	2/2	0/2	0/2	1/2
Urinary (10)	90%	70%	50%	100%	40%	30%	20%
Others (17)	94%	65%	71%	82%	41%	18%	12%
Surgical (50)	94%	50%	48%	68%	24%	38%	12%
All (235)	**93.6%**	**64.3%**	**61.7%**	**79.6%**	**40.4%**	**23.0%**	**12.8%**

**Figures having a denominator lower than 10 are not expressed as percentages.*

(11), fluoroquinolones ranked second after beta-lactams. Lastly, in Australia (12), the most commonly used antimicrobials following amoxicillin with clavulanic acid were trimethoprim-sulphonamides, metronidazole, and fluoroquinolones. Our data in Madrid ranked fluoroquinolones and imidazole derivatives after beta-lactams.

Metronidazole was the most systemically used antimicrobial for enteritis/gastroenteritis in our study, in agreement with the European data of De Briyne and others (2014) (17), but not in Finland (10) or Italy (11). Digestive disorders were the most frequently recorded condition for metronidazole in all these studies, which is in agreement with the guidelines mentioned above (13, 15). According to the Swedish (15) and Danish (13) guidelines, there are few indications for antibiotic treatment of gastrointestinal diseases (such as acute haemorrhagic diarrhea, small intestinal bacterial overgrowth or antibiotic-responsive diarrhea), suggesting that most of these antimicrobial treatments should have been avoided in our surveyed sample.

Aminoglycosides, especially neomycin and tobramycin, were the most commonly used topical antimicrobial class in our study, mainly for treating eye and cutaneous conditions. However, the antimicrobials recommended as the first option for conjunctivitis are fusidic acid (13, 15), polymyxin, and oxytetracycline (for gramnegative rods) and erythromycin (for streptococci) (14).

Another common antimicrobial topical treatment is for ear infections, although the Swedish guidelines (15) recommend that "antibiotics should not be used to treat otitis conditions that are not actually infected with bacteria." A similar approach is followed in the Danish guidelines (13) that only recommend antimicrobial therapy for bacterial-caused otitis externa and otitis media. In our survey, topical polymyxin B was the most widely used, followed by fluoroquinolones (topical and oral formulations), topical florfenicol and oral beta- lactams. Fluoroquinolones were the systemic antimicrobial most frequently used for ear infection in dogs and cats in Italy (11).

Although in our survey fluoroquinolones were not the most frequently used antimicrobial for any condition, the overall data ranked fluoroquinolones as the second most frequently used antimicrobial class. In Europe (17), fluoroquinolones ranked second for skin and genitourinary infections and third for respiratory diseases in dogs, but the situation certainly varies among countries. In Italy (11), data from dogs and cats together, fluoroquinolones were ranked second after beta-lactams (penicillins and cephalosporins). In the UK (8), the use of fluoroquinolones was lower than the use of beta-lactams, nitroimidazoles, and lincosamides. In Finland (10), use of fluoroquinolones was less than beta-lactams, trimethoprim-sulphonamides and macrolide and lincosamides.

These data highlight that certain antimicrobial classes are preferred in certain countries (17), which might be related to interlinked factors such as differences in the prevalence of diseases, antimicrobial resistance levels, existing guidelines on antibiotic prescription, authorized VMP or prescribing behavior.

Amoxicillin with clavulanic acid and fluoroquinolones are good examples of broad-spectrum antibiotics. Some authors

(7, 12) believe that their high use suggests a low standard of diagnosis by the clinician. The infrequent use of bacterial culture and antimicrobial susceptibility testing found in our study has also been previously emphasized (3, 11, 19) and could be one of the reasons for the high use of broad-spectrum antibiotics for empiric treatments [Escher et al. (11)]. According to different authors (2, 3, 19), antimicrobial therapy based on antimicrobial susceptibility testing is mainly reserved for complicated cases or after a preliminary poor response. Equally, cytology [the "microscopic examination of smears of exudates or aspirates from the infected site"(12)] is another easy and valuable diagnostic tool for bacterial infection (12, 13, 15), rarely used according to our survey.

De Briyne et al. (3) analyzed information sources guiding antibiotic prescription across Europe showing that companion animal practitioners, apart from the Swedish, as well as colleagues within the food production sector, do not consider guidelines as among the most important sources. Indeed, among EU countries we only found guidelines in the English language for antibiotic prescription in companion animals from Sweden (15) and Denmark (13), as mentioned before. Guidelines from other countries, such as Australia (20), are also available. In addition, there are also specific guidelines [respiratory tract infections (21), urinary tract diseases (22), and superficial bacterial folliculitis (23)] of the Working Group of the International Society for Companion Animal Infectious Diseases, a chart with recommendations of the Federation of European Companion Animal Veterinary Associations (24) and those of Guardabassi and others (2008) in a book (14).

Differences between countries in the prevalence of the main bacterial infections in dogs (where antimicrobials are the first therapeutic option) are not documented but do not appear to be a major factor contributing to the dissimilarities in antimicrobial prescriptions between countries.

Surprisingly for us, we found few reports concerning the most common conditions treated with antimicrobials in dogs. Nonetheless, the uncertainties that we observed when studying the information on medical records helped us to appreciate the difficulties in coming to a proper diagnosis. Manual checking of clinical databases (7) confirmed the difficulties in obtaining a final diagnosis by veterinary surgeons and the need for a standardized nomenclature for recording clinical diagnoses.

Perioperative antimicrobial prescription, before, during or after surgical procedures (15) is also a controversial subject. An article from the USA (25) focuses the subject on the decreasing incidence of surgical site infection by the implementation of appropriate antimicrobial therapy. Whereas the Swedish guidelines (15) are highly restrictive and only recommend prophylactic antimicrobial prescription in the cases of dirty wounds, contaminated wounds "if the risk of infection is deemed to be considerable," clean-contaminated wounds "if the operation is estimated to last more than one and a half to two hours" and in a short list of operations. The Danish guidelines (13) emphasize the dog's status and expected surgery as the main criteria and recommend that only high-risk patients should receive antibiotics [those having serious or life-threatening systemic diseases and those who are not expected to survive 24 hours without surgery (13)]. Rantala et al. (10) found that 12% of the prescriptions in their study were for postoperative treatment, while in our survey the figure was quite similar (9.4%), although most uses did not fulfill the Swedish guidelines (15).

Our results revealed a noticeable off-label use of VMP in dogs, mainly related to failure to comply with the SPC on dosage and indication of use. In addition, we detected the use of human products in 37.2% of cases that probably would not be entirely supported by the cascade procedure. Most of the conditions described have a veterinary product authorized for dogs in Spain. According to an EMA reflection paper (5), the proportion of use of human products in cats and dogs ranges from 13 to 80%, but it is not clear if the same procedure for assessing off-label use was applied in all surveys. For instance, Escher et al. (11) reported off-label use with regard to the species' indication (dogs or cats) in 23.8% of cases, most of them because of labeling of the product for human use. Compliance with the dosage recommended by the manufacturer (±20%) was 53.4%. Our finding regarding higher overdosage than underdosage has also been previously reported (16). Nevertheless, this estimation may be markedly skewed because of the comparison only with SPC and not current guidelines.

A different issue arises when both the veterinary and human products have exactly the same active substance and comparable indications for use in animals and humans. According to **Table 1**, there are several drugs where practitioners could prescribe either veterinary or human products but choose the human product because of its lower price as mentioned by Escher et al. in Italy (11). Although these should be considered as examples of off-label use, in our opinion the risk of encouraging antimicrobial resistant bacteria does not change if the active substance is the same and the posology is correctly adapted for dogs.

Our results confirm that a selective pressure for antimicrobial resistant bacteria in dogs is operating in the city of Madrid, which could increase the risk for owners and workers of colonization or even infection with resistant bacteria from pets (2). Potential measures to mitigate this risk would be the improvement of the prescription controls for antimicrobials by veterinary practitioners, reducing empiric treatments and promoting better use of hygiene measures (hands washing) for owners after every contact with animals.

In conclusion, although surveys in other Spanish cities are needed to confirm our findings, the pattern of antimicrobial prescription in dogs in our study is similar to that described in several European countries, and encompass the same two highly interconnected key features: a very high level of use of amoxicillin with clavulanic acid and a very low level of antibiotic sensitivity testing. Consequently, attempts should be made to improve both features at the same time. The feasibility of antibiotic sensitivity testing depends on the promptness of results and price, as well as on the promotion of its usefulness for everyday practice. Increased use of antibiotic sensitivity testing could potentially reduce the empiric prescription of broad-spectrum antimicrobials, such as amoxicillin with clavulanic acid or fluoroquinolones, in favor of other equally effective antimicrobials but less risky for public health.

AUTHOR CONTRIBUTIONS

BG-P performed sampling and data analysis and revised the drafted manuscript. MM designed the study, revised the analysis of data, and drafted manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2018.00309/full#supplementary-material

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Used Daily Dose vs. Defined Daily Dose—Contrasting Two Different Methods to Measure Antibiotic Consumption at the Farm Level

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Tackling the problem of rising antibiotic resistance requires valid and comparable data on the use of antimicrobial drugs in livestock. To date, no harmonized monitoring of antimicrobial usage in animals is available, and there is no system to assess usage data throughout Europe, thus hampering a direct comparison between different European countries. Most of the currently applied monitoring systems are based on sales data. Placement of sales data in relation to the population at risk requires overall assumptions about the weights of the animals treated and the doses applied. Only a few monitoring systems collect data in which the number of treated animals is reported exactly and does not need to be estimated. To evaluate the influence of different calculation methods on the standardizing procedure of antibiotic usage and benchmarking of farms, the treatment frequency for several farms (broiler, suckling piglets, and fattening pigs) was calculated in the following two different ways: first, based on the Used Daily Dose (TF_{UDD}), and second, based on the Defined Daily Dose (TF_{DDD}). To support this evaluation, consumption data from the Veterinary Consumption of Antibiotics Sentinel (VetCAb-S) project in Germany were used as example data. The results show discrepancies between both outcomes depending on the calculation method applied. In broiler holdings, the median values of TF_{DDD} were 20.89% lower than the median values of TF_{UDD}. In suckling piglets and fattening pig holdings, the median values of TF_{DDD} were increased 77.14% and 16.33%, respectively, which may have serious implications for the benchmarking of farms. Furthermore, this finding reflects that the calculation procedure also has an impact on the comparison between populations. Therefore, UDD-based calculations should be preferred to run monitoring systems with a benchmark mission. If, in contrast, the DDD approach is chosen to compare antimicrobial usage between populations, additional considerations should be made to adjust for the addressed discrepancies.

Keywords: defined daily dose, livestock, treatment frequency, treatment incidence, used daily dose

INTRODUCTION

Antibiotic resistance is one of the greatest threats to global health in our century. It can affect anyone, recognizes no borders and leads to higher medical costs, prolonged hospital stays, and increased mortality (1, 2). Although antibiotic resistance evolved long before naturally occurring antibiotics and their derivatives were used to treat human and animal diseases (3), the widespread use of antibiotics in human and veterinary medicine leads to a selective pressure and accelerates this process (4). A central point in establishing an effective strategy to contain antimicrobial resistance in the veterinary sector is to collect and understand data on the consumption of antimicrobials in animals (5). Therefore, standardized indicators of antibiotic usage as well as robust antibiotic monitoring systems are needed. Various indicators are applied to describe antibiotic usage in livestock, the outcomes of which differ and are not always directly comparable (6–8). Currently, no harmonized monitoring system across Europe for antibiotic usage or the assessment of antibiotic usage data exist (9).

Most national reports on antibiotic usage in livestock are currently based on sales data. Sales data are easily available, but they do not provide any information about the treated species, the treatment indication, the number of animals treated or the treatment duration. Evaluating sales data without relation to the potential population at risk and without taking into account the potency and the formulation of drugs has clear limitations (10, 11). There have been several attempts to standardize sales data by taking into account estimates about the treated population to enable comparisons between countries or populations (12, 13).

At the level of the European Union (EU), ESVAC (European Surveillance of Veterinary Antimicrobial Consumption) reports on sales data from 29 EU countries are published annually. In those reports, sales data are harmonized by the animal population by setting the Population Correction Unit (PCU) as a proxy for the animal population at risk in each country. For this calculation, the population at risk of being treated is approximated by the product of the number of individuals at risk of being treated and a standard body weight at treatment (14). The consumption of veterinary antimicrobials is reported in milligrams of active substance per PCU (mg/PCU). Until now, ESVAC has not collected species-specific antimicrobial usage data, and therefore, reports encompass all food-producing animals together, recapped as PCU, precluding the distinction of differences in dosing between species (14).

To enable a more detailed analysis of trends in antimicrobial consumption, ESVAC is striving for the collection of harmonized data on consumption by animal species, as well as a more harmonized calculation method (5). Therefore, "defined daily dose for animals" (DDD_{vet}) and "defined course dose for animals" (DCD_{vet}) values were established for antimicrobials used in the three major food-producing animal species: pigs, cattle and poultry (broiler) (15). The concept of the Defined Daily Dose for Animals (ADD) was first developed by Jensen et al. (11) and is based on the DDD in humans, where DDD is the assumed average maintenance dose per day for a drug used for its main indication in adult persons at 70 kg body weight. Hence, in humans as well as in the veterinary sector, Defined Daily Doses are nearly always a compromise based on a review of available information, such as recommendations on the Summary of Product Characteristics (SPCs) from different countries (10, 11, 15).

Some European countries, such as The Netherlands and Denmark, have also implemented benchmarking systems at the national level based on the DDD concept (16–19). The Defined Daily Doses used in those benchmarking systems were established at the national level and are not based on the DDD_{vet} published by ESVAC (17).

In Germany, in contrast, the Used Daily Dose (nUDD) number per animal directly calculated from the recoded information is applied for benchmarking at the herd level. Other systems utilize a different approach, where the UDD describes the amount of active substance actually administered to the treated animals in mg/kg (20). In contrast to DDD, UDD can only be calculated if the amount of active substance but also the number of treated animals as well as the number of treatment days, is recorded (21). Since the German Medicinal Products Act entered into force in 2014, feedlots for fattening pigs, calves and cattle for meat production and fattening poultry (chicken and turkeys) are required to submit detailed information about each antibiotic treatment and the number of animals kept (22). The treatment frequency (TF) was set up as the benchmarking indicator. Calculation of the TF for all farms separated by species and age group is performed twice a year and officially published by the Federal Office of Consumer Protection and Food Safety. The median and 75% percentile of the TF distribution are defined to be specific benchmark thresholds in this system, as determined for areas with legal regulated actions (see **Figure 1**) (22).

In our current evaluation of a data subset of the VetCAb-S study collective (23), we aimed to compare two different methods that are used to calculate antibiotic usage at the farm level. We investigated the differences between applying the Used Daily Dose (UDD) and Defined Daily Dose (DDD) to quantify antibiotic consumption and benchmark farms. The aim of the study was to demonstrate the discrepancies between the outcomes of both methods and their impact on the German benchmarking system. We hope that the outcomes of this work can be used as guidance in implementing, evaluating or improving antibiotic usage monitoring systems in livestock at the bottom-up level. Therefore, we calculated the TF based on

Abbreviations: ADD, Defined Daily Dose Animal; ADF, Application and Delivery Form; DCD_{vet}, Defined Course Dose for animals; DDD, Defined Daily Dose; DDD_{vet}, Defined Daily Dose for animals; EMA, European Medicines Agency; ESVAC, European Surveillance of Veterinary Antimicrobial Consumption; EU, European Union; KTBL, Kuratorium für Technik und Bauwesen in der Landwirtschaft e. V.; nDDD, Number of Defined Daily Doses; nUDD, Number of Used Daily Doses; PCU, Population Correction Unit; QS, QS Qualität und Sicherheit GmbH; SPC, Summary of Product Characteristics; TI, Treatment Incidence; TI_{UDDpig}, Treatment Incidence based on Used Daily Dose in pigs; TI_{ADDpig}, Treatment Incidence based on Animal Daily Dose in pigs; TF, Treatment Frequency; TF_{DDD}, Treatment Frequency based on Defined Daily Dose; TF_{UDD}, Treatment Frequency based on Used Daily Dose; UDD, Used Daily Dose; UDD_{pig}, Used Daily Dose in pigs; VetCAb-S, Veterinary Consumption of Antibiotics Sentinel.

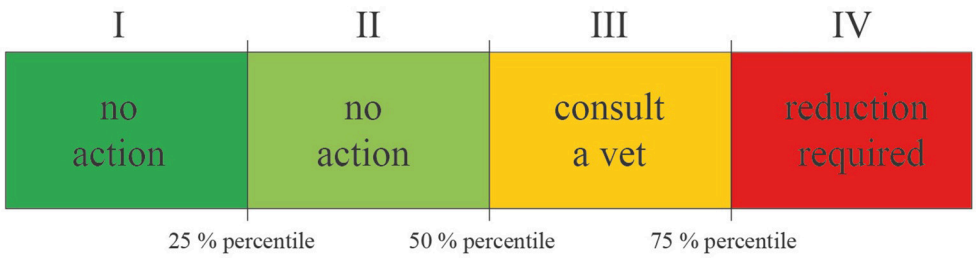

FIGURE 1 | Benchmarking areas of the treatment frequency according to the requirements of the German Medicinal Products Act.

the nUDD (TF_{UDD}), with knowledge of the number of animals treated as well as the treatment duration, following a calculation method very similar to the calculation method established in the German Medicinal Products Act, where only the reference population in the denominator slightly differs. For all these treatment records, we also calculated the TF based on the nDDD (TF_{DDD}) by estimating the number of animals treated, considering the amount of active substance delivered to the farmer or applied by the veterinarian and using assumed standard body weights fixed for animals in this production period.

MATERIALS AND METHODS

Study Data

The VetCAb study started in 2008 as a feasibility project to investigate the practicality of implementing an antibiotic monitoring system under the conditions of the German veterinary and agricultural system (24). In 2011, a pilot project was carried out as a cross-sectional study including nearly 3,000 animal holdings across the country (25). Since 2013, the study was continued using a longitudinal approach as VetCAb-Sentinel (VetCAb-S). The study population consists of an open cohort with ongoing participant recruitment, designed to provide a stable study size over time (23). Data collection is related to the mandatory documentation in application and delivery forms (ADF), which is legally required by the German Medicinal Products Act and delivered by farmers or veterinarians to the VetCAb database. These forms include information about the animal species and the number of animals treated, the treatment or delivery date, the treatment indication and application route, the name and amount of the antimicrobial drug applied and, respectively, delivered, and information about the duration of the treatment (26).

From the ongoing VetCAb-S project (23), data on 40 broilers, 135 suckling piglets and 449 fattening pig farms, which participated in the study in 2014, were included in this evaluation. During the time period surveyed, 5% of the broiler farms did not use antibiotics at all. No antibiotic usage was observed in 13.3% of the suckling piglet farms and 14.5% of the fattening pig farms. The treatment frequency was calculated based on the Used Daily Dose (TF_{UDD}) following the rules of the German Medicinal Products act and the Defined Daily Dose (TF_{DDD}), using DDD_{vet} assigned by ESVAC for pigs and broilers for every active compound and application route (27). Because DDD_{vet} for broilers and pigs were only determined for the oral (broiler) or the oral and parenteral application routes (pigs), respectively, we limited the analyzed dataset exclusively for records of oral and parenteral treatments. Hence, the median of the TF calculated in this particular evaluation may vary from previously published TF where other application routes were also included.

Treatment Frequency

The treatment frequency is an indicator of the antimicrobial usage in livestock at the farm level, and in Germany it is used as an indicator in the benchmarking system. The TF indicates for how many days, on average, an animal in the observed population is treated within a given time period, e.g., how many single doses were administered to one animal on average within the observation period (21). It describes the number of treatment days per given time period and farm. The treatment frequency meets the classic definition of an incidence of contrasting events in a given population at risk within a defined time period (28).

Within the German benchmarking system, the TF is calculated twice a year according to the following Equation (1) (22):

$$TF = \frac{\#\ animals\ treated\ \times\ \#\ treatment\ days\ \times\ \#\ active\ compounds}{\#\ animals\ in\ the\ population} \quad (1)$$

This calculation method considers the actual number of animals treated, the treatment duration and the number of active compounds in the numerator, and the actual number of animals in the entire farm population in the denominator. The number of active compound depends on the veterinary medicinal product used. Mono-preparations contain only one antimicrobial active ingredient, while combination products contain two or more active substances. Therefore, treatments performed in the same number of animals for the same treatment duration lead to a two-fold higher TF if a combination product, such as sulfonamide/trimethoprim combination treatment, is used [see Equation (1)].

In Equation (1), the amounts used, doses or body weights are only considered indirectly. To include those variables in the calculation, a rearrangement of Equation (1) is needed [see Equation (3) and Equation (4)].

Used Daily Dose and Treatment Frequency

The Used Daily Dose (UDD) is defined as the actual administered dose per actual kg animal per day. The UDD (mg/kg) can differ between herds and treated animals and must be calculated for every treatment separately (21, 29). In contrast to (1), calculating

the UDD in mg/kg requires knowledge concerning the amount of active substance delivered to the farmer, the number and weight of animals treated and the treatment duration (21, 29, 30), as outlined in Equation (2).

$$UDD\left(\frac{mg}{kg}\right) = \frac{amount\ of\ active\ substance\ (mg)}{\#\ animals\ treated\ \times\ animal\ weight\left(kg\right) \times\ \#\ treatment\ days} \quad (2)$$

Taking into account the amount of antibiotics used, the body weight of the animals treated and the dosage applied, Equation (1) can be rearranged to Equation (3) (30):

$$TF_{UDD} = \frac{amount\ of\ active\ substance\ for\ every\ active\ compound\ (mg)}{\#\ animals\ in\ the\ population\ \times\ animal\ weight\left(kg\right)\ \times\ UDD\left(\frac{mg}{kg}\right)} \quad (3)$$

Hence, the TF_{UDD} calculation method in (3) corresponds to the calculation method for the treatment frequency as shown in (1) and currently applied within the German benchmarking system as laid down by the German Medicinal Products Act (22).

In this paper, the number of livestock places was used in the denominator as a proxy for the animals in the population to calculate the treatment frequency (6, 23, 24, 26). Therefore, the TF_{UDD} as well as the TF_{DDD} calculated within this evaluation indicate how many single doses were administered per livestock place per given time period and farm. Livestock places for piglets were calculated by multiplying the number of livestock places for sows by 10.25, which is the average number of piglets per litter in Germany (23, 31).

Defined Daily Dose and Treatment Frequency

The Defined Daily Dose (DDD) is the assumed average dose per kg animal per species per day (11, 15). Within monitoring systems, in which antibiotic usage reporting is based on the amount of active substance (16, 18), there is no information about the number of animals treated, and treatment duration or the daily dose actually applied is provided, the treatment frequency can only be estimated by applying standard body weights and Defined Daily Doses, yielding Equation (4).

$$TF_{DDD} = \frac{amount\ of\ active\ substance\ for\ every\ active\ compound\ (mg)}{\#\ animals\ in\ the\ population\ \times\ standard\ animal\ weight\ \left(kg\right)\ \times\ DDDvet\left(\frac{mg}{kg}\right)} \quad (4)$$

In (4), the number of single doses is estimated by considering the amount of active substance delivered to the farmer standardized by DDD_{vet} and the standard weights of the animals treated. The standard weights considered for the TF_{DDD} calculation correspond to the standard weight proposed by ESVAC (5) and are as follows: suckling piglets (standard weight 4 kg), fattening pigs (standard weight 50 kg) and broilers (standard weight 1 kg). The DDD_{vet} published by ESVAC in April 2016 for pigs and broilers (27) was used for the evaluation. DDD_{vet} is a technical unit and defined to be the assumed average dose per kg animal per species per day (mg/kg), taking into account differences in the dosing, pharmaceutical form and application route used in different species (15). Data on dosing (daily dose and number of days of treatment recommended for the main indication) obtained from the SPCs for antimicrobial veterinary medicinal products were provided for broilers, cattle and pigs by nine EU member states to ESVAC. DDD_{vet} were calculated as the average of all observations of daily doses by species, substance and form (15). As the DDD_{vet} for three long-acting macrolid injectable products, namely, gamithromycin, tildipirosin and tulathromycin, have not yet been published, we set up the DDD based on the Summaries of Product Characteristics of veterinary medicinal products containing these active substances, considering veterinary medicinal products that are only licensed in Germany. Defined Daily Doses were set up as follows: gamithromycin 6 mg/kg, tildipirosin 4 mg/kg and tulathromycin 2.5 mg/kg.

Benchmarking

To describe the distribution of the TF within the population of farms, the 25% percentile, median and 75% percentile were set as specific benchmark thresholds, resulting in four distribution areas of action (dark and light green: no action, yellow: veterinary consulting useful, red: reduction required, see **Figure 1**) corresponding to the requirements of § 58 of the German Medicinal Products Act (22). To identify differences in calculation methods, we compared both TF distributions to identify the number of farms in which differences between both outcomes resulted into a shift between action areas within the scope of the German benchmarking system. To demonstrate these differences, cumulative distribution functions were used to show the shift in location and the shape of the distribution. In addition, similarity matrices were employed to describe the number of concordant and discordant results for both measures.

Estimated Animal Weight at the Time of Treatment

In Germany, the weight of the animals at the time of treatment is not recorded in the ADF forms. Therefore, we calculated the weight of the animals treated for every record following a rearrangement of Equation (2), see Equation (5). In this case, we assumed that the UDD (mg/kg) was the recommended dosage in the SPCs of every veterinary medical product used in the dataset evaluated. For every veterinary medical product used in this evaluation, therefore, we calculated the recommended dosage in mg/kg derived from VETIDATA, a specialized German information platform on questions regarding the usage of medicinal products, toxicology and the legal framework on medicinal products in veterinary medicine (www.vetidata.de).

Information on the amount of active substance, number of animals treated and treatment days is mandatory in ADF forms.

$$\text{animal weight}\ \left(\text{kg}\right) = \frac{\text{amount of active substance (mg)}}{\#\ \text{animals treated} \times \text{UDD}\left(\frac{\text{mg}}{\text{kg}}\right) \times \#\ \text{treatment days}} \quad (5)$$

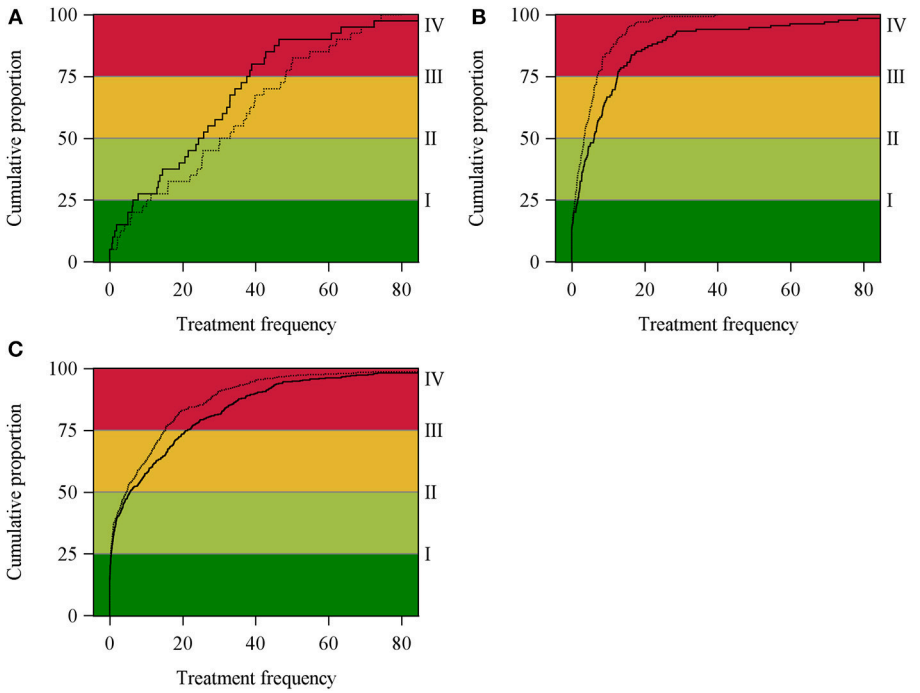

FIGURE 2 | Cumulative distribution function of TF_{UDD} (dashed line) and TF_{DDD} (solid line) in broiler **(A)**, suckling piglets **(B)** and fattening pig **(C)** holdings.

All the statistical evaluations mentioned above were performed with SAS®, version 9.3 TS level 1M2 (SAS Institute Inc., Cary, NC, United States). The graphical representation of the cumulative distribution function of TF_{UDD} and TF_{DDD} shown in **Figure 2** was created using the SAS procedure proc univariate.

RESULTS

Distribution of Treatment Frequencies Due to the UDD vs. DDD Calculation

Treatment frequencies were calculated for each animal holding following the UDD- and the DDD-concept, respectively (see **Table 1**). The median of the TF_{UDD} of all suckling piglet holdings was 3.4 with a maximum of 39.3. In the fattening pig holdings, the median of the TF_{UDD} was 4.7. In broiler holdings, the median of TF_{UDD} was 31.6. Based on the DDD, the median of the TF_{DDD} in broiler holdings was 25, and in suckling piglets and fattening pig holdings, it was 6.2 and 5.6, respectively.

Cumulative distribution functions of the TF_{UDD} and TF_{DDD} for broiler, suckling piglets and fattening pig holdings are shown in **Figure 2**. In broiler holdings (a), the cumulative distribution function of TF_{DDD} generally runs above the cumulative distribution function of TF_{UDD}. Within the upper quarter of the distributions, crossing functions are observed indicating substantial differences in the measurements. In contrast, in suckling piglets (b) and fattening pig holdings (c), the cumulative distribution function of TF_{UDD} covers almost the cumulative distribution function of TF_{DDD} in the lower 50% of the data and runs above the function of TF_{DDD} in the upper 50% of the records (see **Figures 2A–C**).

Similarity of Benchmarking due to UDD- vs. DDD-Calculation

To demonstrate the shift in both distributions for all species/age groups considered, a similarity matrix for the four areas of action was calculated, showing concordance and discordance in these benchmark areas (see **Tables 2–4**).

In broiler farms, we found the highest discordance among all evaluated production groups. An overall similarity of only 50% indicates a high percentage of farms shifting between categories. Given that neither the first (dark green) nor the second category (light green) are legally restricted, shifts between those categories will not have any consequences for the farmer (**Figure 1**). This outcome looks different in those cases where there are shifts in or between the third (yellow) and fourth (red) category. In total, 50% of all farms classified to be in the third category (yellow) using the UDD to calculate the TF no longer remained therein using TF_{DDD}. Additionally, 20% of those farms shifted into the fourth category (red), and according to the regulations of the German Medicinal Product Act, the development of an action plan would become mandatory for these farms. Finally, 30% shifted into the second category and were no longer subject to any legal regulations (see **Table 2**).

In 34.1% of all evaluated suckling piglets holdings, there was no match between the categories of TF_{UDD} and TF_{DDD}. The highest similarity in benchmarking was found in the first TF category, where only 12.1% of the farms shifted to another

TABLE 1 | Summary of the treatment frequency for broilers, suckling piglets and fattening pigs based on UDD and DDD.

Species/age group	Number of holdings	Minimum	5%-Percentile	Median	Upper quartile	95%-Percentile	Maximum
TF_{UDD}							
Broilers	40	0	1.0	31.6	48.2	70.7	74.4
Suckling piglets	135	0	0	3.4	7.1	15.8	39.3
Fattening pigs	449	0	0	4.7	15.2	40.0	409.3
TF_{DDD}							
Broilers	40	0	0.3	25	38	67.9	98.4
Suckling piglets	135	0	0	6.2	12.6	54.5	101.7
Fattening pigs	449	0	0	5.6	21.3	52.1	613.9

TABLE 2 | Similarity in benchmarking due to TF_{UDD}- and TF_{DDD}-distributions for broilers (overall similarity 50%).

TF_{UDD}	TF_{DDD}							
	I		II		III		IV	
	n	%	n	%	n	%	n	%
I	8	80	2	20	0	0	0	0
II	2	20	3	30	1	10	4	40
III	0	0	3	30	5	50	2	20
IV	0	0	2	20	4	40	4	40

Dark and light green category, no action needed; yellow category, veterinary consulting useful; red category, reduction required.

TABLE 3 | Similarity in benchmarking due to TF_{UDD}- and TF_{DDD}-distributions for suckling piglets (overall similarity 65.9%).

TF_{UDD}	TF_{DDD}							
	I		II		III		IV	
	n	%	*n*	%	*n*	%	*n*	%
I	29	87.9	4	12.1	0	0	0	0
II	4	11.8	20	58.8	9	26.5	1	2.9
III	0	0	9	26.5	16	47.1	9	26.5
IV	0	0	1	2.9	9	26.5	24	70.6

Dark and light green category, no action needed; yellow category, veterinary consulting useful; red category, reduction required.

TABLE 4 | Similarity in benchmarking due to TF_{UDD}- and TF_{DDD}-distributions for fattening pigs (overall similarity 80.4%).

TF_{UDD}	TF_{DDD}							
	I		II		III		IV	
	n	%	*n*	%	*n*	%	*n*	%
I	105	93.8	7	6.3	0	0	0	0
II	7	6.3	89	79.5	16	14.3	0	0
III	0	0.9	16	14.3	75	67	21	18.8
IV	0	0	0	0	21	18.6	92	81.4

Dark and light green category, no action needed; yellow category, veterinary consulting useful; red category, reduction required.

category with no legal consequences for the farmer. The lowest similarity was found in the third category, where only 47.1% of the farms remained in the same category if DDD was used (see **Table 3**).

In the group of fattening pigs, we found the highest concordance over all evaluated production groups in the benchmarking of farms (overall similarity 80.4%). We observed 93.8% (dark green), 79.5% (light green), 67% (yellow), and 81.4% (red) similarity in benchmarking for the first, second, third and fourth category, respectively (see **Table 4**).

Distribution of the Estimated Animal Weight at the Time of Treatment

Figure 3 shows the distribution of the calculated weight for broilers (A), suckling piglets (B) and fattening pigs (C) based on

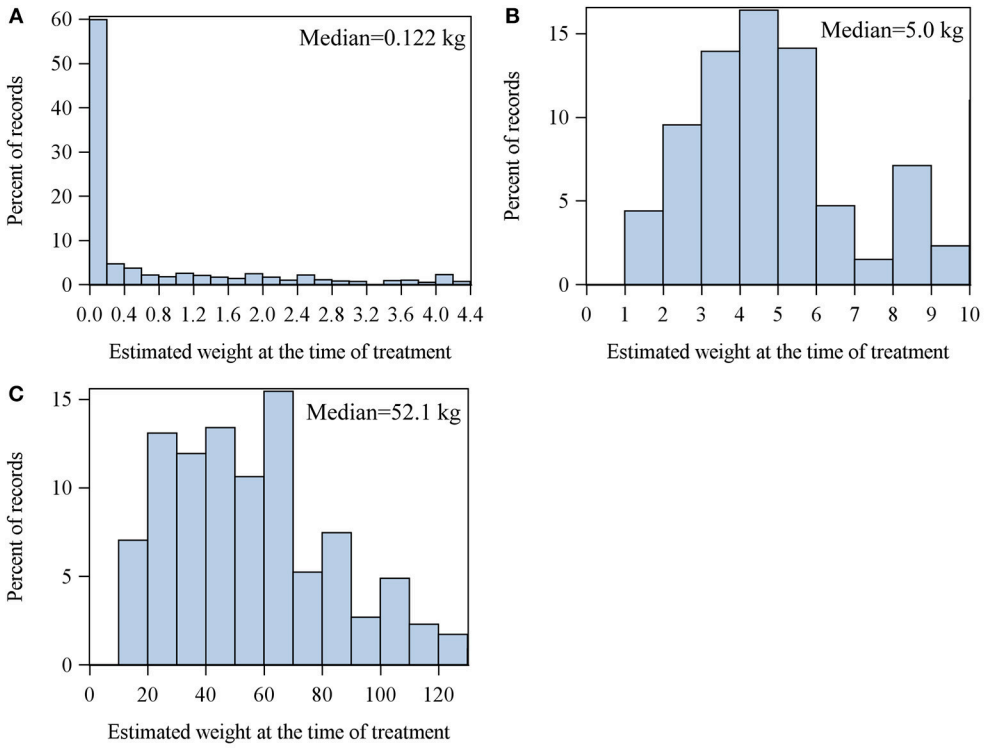

FIGURE 3 | Distribution of the estimated weights of broiler **(A)**, suckling piglets **(B)** and fattening pigs **(C)** at the time of treatment.

the ADFs considered in this current evaluation. The median of the estimated weight of the broilers was 0.122 kg, suckling piglets 5 kg and fattening pigs 52.083 kg.

DISCUSSION

The present work compares two different methods to calculate antibiotic usage in livestock, demonstrating the differences between applying the Used Daily Dose (UDD) and Defined Daily Dose (DDD) and their consequences for individual farmers as well as at the general population level. Both TF calculations are generally in line with the incidence density concept for presenting new events within a given time period (28).

In this evaluation, we used the number of livestock places as a proxy for the animal population at risk (23, 24, 26). The number of livestock places is not exactly equal to the number of animals stabled and maintained during the fattening period, which could vary slightly due to mortality or temporary overcrowding. Those differences between livestock places and the exact number of animas stabled (or present at the farm at any time) can lead to an over- or underestimation of the TF at some point, but we consider that bias to be negligible and compensated by the observation that the number of barn places remains stable over time. In particular, information bias due to under- or misreporting of the number of animals that were stabled or that died during the fattening period can be minimized. Therefore, the number livestock places is more precise and general bias is restricted. This denominator also indirectly considers the observation that there is more than one flock/batch kept per year. The number of treatment days per flock or batch, respectively, could be calculated by dividing the TF calculated per year by the number of flocks/batches per year.

In broiler holdings, the median values of TF_{DDD} were 20.89% lower than the median values of TF_{UDD}, while in suckling piglets and fattening pig holdings, the median values were 77.14% and 16.33% higher, respectively. Additionally, the cumulative distribution functions showed similar differences in the shape distributions of TF_{UDD} and TF_{DDD}.

Regarding the benchmarking of farms, in 50% of broiler holdings, 34.1% of suckling piglet holdings and 19.6% of fattening pig holdings, the different calculation methods resulted in a shift to another category, potentially associated with varying legal obligations for the farmers.

In a study with a similar approach, Timmerman et al. (29), compared the treatment incidence based on UDD_{pig} (TI_{UDDpig}) and ADD_{pig} (TI_{ADDpig}) in pigs and found TI_{ADDpig} to be higher than TI_{UDDpig}. In this study, ADD_{pig} was estimated based on national dose recommendations from two sources regularly consulted by Belgian veterinarians. The authors considered the discrepancies between TI_{ADDpig} and TI_{UDDpig} to be mainly a consequence of inappropriate dosing, misinterpretations of the leaflet instructions or incorrect evaluations of body weights. Persoons et al. (20), compared TI based on UDD with TI based

on DDD in Belgian broiler farms and concluded that, based on UDD, fewer chickens per 1,000 chickens at risk per day were treated than theoretically expected when applying DDD.

Mathematically, differences in both outcomes of the TF are the result of different numbers of single doses used to calculate the TF. By calculating the number of single doses, the amount of active substance (mg) in the nominator always remains the same, regardless of whether the calculation is based on UDD or on DDD. In contrast, the weight of the treated animals and the daily dose considered in the numerator are subject to change, resulting in differences in the number of single doses. Therefore, discrepancies between TF_{UDD} and TF_{DDD} exist for two reasons: primarily, the weight of the treated animals at the time of treatment, considered by calculating TF_{UDD}, is not always equal to the standard weight used to calculate TF_{DDD}, and second, because UDD is not necessarily equal to DDD.

The weight of animals varies considerably in farming practice. In Germany, broilers are stabled at the age of 1 day (ranging from 1 to 3 days) with a body weight of 40 g (ranging from 38 g to 45 g) and leave for slaughter at the age of 32 to 40 days with an end weight of 1.6 to 2.4 kg. Suckling piglets have a birth weight of 1.5 kg (ranging from 1 to 1.7 kg) and reach 6.9 kg (21-day suckling period) or 8.1 kg (28-day suckling period), respectively, at the time of weaning (ranging from 5.8 kg to 8.8 kg). Fattening pigs are stabled with an average weight of 28 kg (ranging from 25 kg to 30 kg) and leave approximately 115 days later for slaughter with an average end weight of 118 kg (ranging from 110 kg to 120 kg) (32).

Generally, the lower the weight of the treated animals compared with the standard weight, the lower is the treatment frequency of the DDD approach, leading to an underestimation of the TF_{DDD}. Conversely, the treatment of animals that are heavier than the standard weight leads to an overestimation of TF_{DDD}.

Our results showed the TF_{DDD} in broilers was 20.89% lower than TF_{UDD}. We consider this underestimation to be mostly due to discrepancies between the standard weight and the real weight of the animals at the time of treatment. The weight of broilers changes by a factor of 40 to 60 during their life span, which carries a high risk of uncertainties in terms of weight estimation. Due to data on treatments in broilers (QS, personnel communication) in Germany, 50% of all treatments take place during the first 7 days of the fattening period, in which the body weight of the animals varies between 40 g and 400 g. In over 70% of the records in our dataset, the weight of the treated broiler was estimated to be <1 kg, likely explaining the underestimation of TF_{DDD} by 20% in relation to TF_{UDD}. Therefore, we consider the main reason for the systematic differences in TF calculations to be due to this bias and the differences between UDD and DDD to be of secondary importance in broilers. In suckling piglets and fattening pigs, in contrast, the distribution of the calculated weights of the animals was more symmetric near the standard weights proposed by ESVAC. In contrast to broilers, the weight of suckling piglets changes only by a factor of 5 to 6 on average between birth and weaning. The weight of fattening pigs during a fattening period changes by the factor of 4 on average. Hence, in pigs, systematic errors due to weight variations were lower than in broilers. However, in the estimation of animal weights at time of treatment, we assumed UDD (mg/kg) to be the recommended dosage derived from the SPCs of every veterinary medical product used. Interpreting the distribution of the estimated weight bias due to under- or overdosing needs to be considered.

In addition to the animal weight at treatment, the difference between DDD and UDD must be taken into account. The DDD_{vet} is the assumed average dose per kg animal per species per day and was assigned as an average of the daily doses obtained from Summaries of Product Characteristics (SPCs) for antimicrobial veterinary medicinal products provided for broilers, cattle and pigs by nine EU countries (15). The observations were based on the main indication. DDD_{vet} is a technical unit of measurement that is solely intended for the purposes of drug consumption studies and does not necessarily reflect the daily doses recommended, prescribed or used by the veterinarian's decision.

In contrast, the UDD is the administered dose per kg animal per day determined at the discretion of the veterinarian and dependent on different criteria, such as the veterinary medical product used, clinical picture, pathogenic agents, progression, and spread of the disease, resistance situation, general condition of the patient, etc. The UDD therefore differs between herds, treated animals and veterinarians, and it needs to be calculated for every treatment scenario separately (21). Generally, UDD can also be represented by a statistical distribution within a population under study.

Additionally, systematic differences are observed because the recommended dosage provided in the SPC may vary for the same active substance in and between countries and licensed veterinary medical products. The DDD assigned to be higher than the actually applied UDD leads to an underestimation of the number of single doses and, consequently, a lower TF_{DDD}. Conversely, calculations based on a DDD lower than UDD lead to an overestimation of the number of single doses and, therefore, a lower TF_{DDD}.

DDD_{vet} for oral and injectable preparations included in the ESVAC document were assigned as an arithmetic mean of all observations for each combination of species, antimicrobial substance and administration route over all products marketed in nine European countries (15). Postma et al. (33), established Defined Daily Dose Animal (DDDA) per active substance and administration route (following the ESVAC approach and using the mean of the recommended dosage for the main indication provided in the SPC) over all veterinary medical products authorized for use in pigs in four European countries (Belgium, France, Germany and Sweden). In their study, (33), found 31 out of 82 unique combinations that showed deviations of >10% from the established consensus DDDA, where most of these products contain tylosin, amoxicillin and doxycycline. Tylosin via the oral application route was the active substance, with the highest difference between the minimum and maximum recommended dosage (1000%).

We compared the recommended dosage based on the SPC for five veterinary medicinal products containing tylosin licensed in Germany for oral medication for pigs as an example. We

found the recommended dosage to vary between 4.5 and 25.7 mg/kg body weight depending on the indication, where a main indication could not be identified. The DDD_{vet} for tylosin in oral preparations for pigs is 12 mg/kg. Treating pigs with 4.5 mg/kg as the recommended dosage and estimating the number of treated animals based on a DDD_{vet} of 12 mg/kg in the DDD approach leads to an underestimation of the number of treated animals by a factor of 2.5. Conversely, using 25.7 mg/kg as the recommended dosage leads to an overestimation of the number of animals treated in the DDD approach. We consider such differences between UDD and DDD to have played the major role in discrepancies between TF_{UDD} and TF_{DDD} in our dataset for pigs.

CONCLUSION

The results of this evaluation show that the variable used to quantify antibiotic usage has a significant impact on the outcome. It has been demonstrated that the UDD is the most suitable indicator in regard to benchmarking of farms because it represents the real situation on the farm and considers the dosage actually applied as well as the weight of the treated animals. Therefore, we recommend using UDD calculations whenever possible to avoid under- or overestimation of antibiotic usage at the farm level. As a consequence, collection systems for antibiotic usage data need to be expanded with additional information, such as the number of treated animals and the treatment duration. In those cases where using UDD is not an option, e.g., if only sales data are available, one should be aware of the risk of under- or overestimation of the number of animals treated, especially if the treated animals do not reach the standard weight or the national dosages applied substantially differ from the proposed DDD. For broilers, we strongly recommend the standard weight of 1 kg to be adjusted downwards, as we could show that most treated animals had a much lower body weight.

ETHICS STATEMENT

In this study, two different calculation methods of the treatment frequency were evaluated. The data used herein were based on mandatory application and delivery forms, and they were provided voluntarily by farmers and veterinarians after signing individual written consent to the use of the data by the study team only. Our research did not involve any regulated animals, and no scientific procedures were performed on animals of any kind. Thus, formal approval by an ethical committee was not necessary under the provisions of German regulations.

AUTHOR CONTRIBUTIONS

SK and LK: conceptualization, formal analysis, investigation, and writing—original draft. SK and MH: data curation. LK: funding acquisition. SK, MH, and LK: methodology. SK: project administration. MH: software. LK: supervision. SK and MH: validation and visualization. SK, NW, AK, and LK: writing—review and editing.

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Antimicrobial Use and Antimicrobial Resistance Indicators—Integration of Farm-Level Surveillance Data From Broiler Chickens and Turkeys in British Columbia, Canada

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Using data from the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS), we aimed to describe trends in antimicrobial use (AMU) in broiler chickens and turkeys, to compare AMU across species, to compare with trends in antimicrobial resistance (AMR), and to assess the effects of various AMU/AMR units of measurement (metrics and indicators) on data integration. Data on AMU and AMR in enteric bacteria, collected from 2013 to 2017 from broiler chickens (n = 143 flocks) and turkeys (n = 145) were used. In broiler chickens, the total AMU in milligrams/population correction unit (mg/PCU_{Br}) decreased by 6%, the number (n) of defined daily doses for animals using Canadian standards (nDDDvetCA) per 1,000 broiler chicken-days decreased by 12%, and nDDDvetCA/PCU decreased by 6%. In turkeys, the mg/PCU_{Tk} decreased by 1%, whereas the nDDDvetCA/1,000 turkey-days and the nDDDvetCA/PCU increased by 1 and 5%, respectively. The types of antimicrobial classes used in both species were similar. Using the frequency of flocks reporting use (i.e., number of flocks reporting use/number of flocks participating) as a measurement, the use of certain antimicrobials changed over time (e.g., Broilers, decreased cephalosporin use, virginiamycin use, emerging use of lincomycin-spectinomycin, and avilamycin; Turkeys: increased trimethoprim-sulfonamides and macrolide use). The trends in resistance to specific antimicrobials paralleled the frequency and quantity of use (e.g., ceftriaxone use decreased—ceftriaxone resistance decreased, and gentamicin use increased—gentamicin resistance increased) in some situations, but not others (decreased fluoroquinolone use—increased ciprofloxacin resistance). AMR data were summarized using the AMR indicator index (AMR Ix). The most notable AMR Ix trend was the decrease in ceftriaxone AMR Ix among *Escherichia coli* (0.19 to 0.07); indicative of the success of the poultry industry action to eliminate the preventive use of third generation cephalosporins. Other trends observed were the increase in ciprofloxacin AMR Ix among *Campylobacter* from 0.23

to 0.41 and gentamicin AMR Ix among *E. coli* from 0.11 to 0.22, suggestive of the persistence/emergence of resistance related to previous and current AMU not captured in our surveillance timeframe. These data highlight the necessity of multiple AMU and AMR indicators for monitoring the impact of stewardship activities and interventions.

Keywords: metrics, indicators, farm-level, surveillance, Canada

INTRODUCTION

Strengthening current surveillance capacities and expertise in antimicrobial use (AMU) and antimicrobial resistance (AMR) is one of the strategic objectives identified in "Tackling Antimicrobial Resistance and Antimicrobial Use: A Pan-Canadian Framework for Action" (1). This effort aligns with the global call to address AMR, such as the World Health Organization's (WHO) Global Action Plan (GAP) on AMR (2), the Food and Agriculture Organization of the United Nation's (FAO) action plan on AMR (3), and the World Organisation for Animal Health's (OIE) strategy on AMR and the prudent use of antimicrobials (4). The tripartite alliance (FAO-OIE-WHO), in the context of "One Health," is jointly addressing emerging threats in the animal-environment-human interface and identified AMR as one of the initial priority areas for collaboration (5). Canada's Framework for Action involves multi-stakeholder engagement and collaboration (both government and industry), to collectively address AMR.

Many countries have established surveillance systems for AMR in food animals (6–9). Similarly, for AMU surveillance, there are many activities at the global, regional, and national levels involving data collection, reporting and development of AMU metrics and indicators. In 2017, the OIE published its 2nd annual report on the use of antimicrobial agents (10), wherein the global data on the quantity of antimicrobials used in animals weighted by biomass, and stratified by region, were reported for the first time (10). In Europe, the European Medicines Agency (EMA)'s European Surveillance for Veterinary Antimicrobial Consumption (ESVAC project) provides guidance for AMU monitoring (11), and AMU data collection and reporting (12). As suggested in the revised ESVAC reflection paper, AMU data should be collected at the farm level to assess temporal trends and understand overall AMU context and impacts of interventions in terms of prudent use/stewardship (13). Collaborative efforts to address AMR, such as the Joint Programming Initiative on Antimicrobial Resistance (JPI-AMR) contribute to the implementation of WHO's GAP (14). One project relevant to AMU surveillance in animals arising from JPI-AMR is the AACTING project (network on quantification, benchmarking, and reporting of veterinary AMU at farm level), which developed a guideline document on AMU data collection and measurements at the farm level (15).

Once national action plans (NAP) have been developed and implemented (16), it is expected that surveillance systems will be progressively strengthened and that comprehensive data (metadata) will be generated, enabling data integration from surveillance programs across sectors to monitor the overall progress of national or regional interventions to address AMR. In June 2017, the European Centre for Disease Control (ECDC), European Food Safety Authority (EFSA), and EMA published their 2nd Joint Interagency Antimicrobial Consumption and Resistance Analysis (JIACRA Report), integrating AMU and AMR data across animal species and in humans (17), followed by the "Joint Scientific Opinion on a list of outcome indicators as regards surveillance of antimicrobial resistance and antimicrobial consumption in humans and food producing animals" (18).

For AMU surveillance, metrics (the technical units of measurement, such as frequency of use) and indicators (an AMU metric in relation to a denominator, such as animal biomass or animal time unit described below) have been developed. Milligrams weighted by population and weight (mg/PCU) is used for reporting national sales and distribution data across countries in the European Union (11). Another AMU indicator is treatment incidence (TI), which pertains to the total number of defined daily doses in animals adjusted for animal-time units (19–21). The number of defined daily doses in animals per PCU is an AMU measurement to monitor AMU sales data in animals (17). Requirements for AMU measurements vary depending on surveillance objectives and include spatial and temporal resolution (frequency on which AMU data are collected), comprehensiveness (capacity to collect usage data from all units in the target population), stability over time, and comparability between populations (22). An AMR indicator is a summarized AMR measurement integrating select AMR data across different bacterial species (e.g., of public health importance) at the national level, aimed at monitoring national and multi-stakeholder stewardship efforts and initiatives to mitigate AMR risks (17). The antimicrobial resistance indicator index (AMR Ix) is a novel AMR indicator, calculated as the percentage of resistance (or susceptibility) to a certain antimicrobial/s, adjusted by PCU (18).

In Canada, CIPARS (Canadian Integrated Program for Antimicrobial Resistance Surveillance) collects, analyses, and communicates trends in AMU and AMR for select bacteria from humans, and food animals along the production continuum (23). The broiler chicken farm component of CIPARS was initiated in 2013 in the major poultry producing provinces in Canada, including British Columbia (BC). In addition to broiler chickens, samples were also collected from the turkey sector of the poultry industry in BC. The farm component was initiated prior to the May 2014 implementation of the first step of the poultry industry AMU strategy, which entailed eliminating the preventive use of Health Canada's Veterinary Drugs Directorate (VDD) Category I antimicrobials (e.g., 3rd generation cephalosporins and fluoroquinolones) (24, 25). Veterinary antimicrobials used in Canada are categorized by Health Canada's Veterinary Drugs

Directorate (VDD) according to their importance to human medicine (VDD Category I-very high importance, Category II-high importance, Category III-medium importance, and VDD Category IV—low importance) (26). The second and third steps of the poultry industry AMU strategy aim to eliminate the preventive use of VDD Category II antimicrobials (e.g., aminoglycosides, streptogramins, macrolides, penicillins, trimethoprim-sulfonamides) by the end of 2018 (broiler chickens and turkeys), and Category III antimicrobials (e.g., bacitracins, tetracyclines, sulfonamides) by the end of 2019 for turkeys and 2020 for broiler chickens (i.e., contingent upon reassessment of this preventive strategy on production metrics and AMR prevalence by the end of 2019 in broiler chickens) (25). The data generated during 2013–2017 enabled analyses of various AMU and AMR metrics and indicators to measure the impact of the initial intervention step by the poultry industry. The objective of this study was to describe AMU trends (2013–2017) in poultry sampled through CIPARS in BC, compare AMU between poultry species, describe AMR over time, and to compare potential AMU and AMR indicators for data integration. This work will inform the selection of AMU and AMR indicators to best monitor the progress of the implementation of industry (24, 25) and government initiatives (e.g., enhanced veterinary oversight, prescription of antimicrobials belonging to VDD Categories I to III) to address AMR (27, 28), and will serve as a reference point in BC to measure the future impacts of the poultry industry's on-going AMU reduction strategy (24, 25).

MATERIALS AND METHODS

Poultry data used in the analysis were collected through CIPARS from BC between 2013 and 2017. From this point forward, poultry refers to combined data from commercial broiler chickens and turkeys, unless indicated otherwise.

Farm and Flock Selection

Prior to farm enrollment, veterinarians participating in the CIPARS farm program administered an informed consent to the producers. Briefly, each year, 30 broiler flocks and 30 turkey flocks in BC were selected for surveillance. This is proportional to the broiler and turkey production profiles of the province (29, 30) compared to the rest of Canada, based on a sampling framework described elsewhere (9, 31). One flock per farm was visited by the veterinarian each year. The participating CIPARS veterinarians ($n = 4$) represented 100% of the poultry veterinary practices in BC. A flock, assigned with a unique code (i.e., identity is known only to the veterinarian), is defined as a group of broiler or turkey birds, hatched and placed in the designated production unit (e.g., floor, pen, barn) approximately the same day. A farm is a registered establishment that may have one or more barns in the premise. For farm selection, veterinarians were instructed to follow certain inclusion and exclusion criteria. The farms must be a commercial quota-holding operation (backyard and small flocks excluded) and compliant to on-farm food safety program (e.g., Safe, Safer, Safest™, the Chicken Farmers of Canada's on-farm food safety assurance program and Turkey Farmers of Canada's On Farm Food Safety Program©) (32, 33). Various production systems (antibiotic-free [ABF], raised without antibiotics [RWA] or organic production) were included but veterinarians were instructed to select the number of flocks proportional to their practice profile. Veterinarians ensured that selected farms were representative of all the Canadian Hatcheries Federation member hatcheries supplying chicks and *poults* and representative of the feed mills supplying feeds in BC, and were geographically distributed across the province (i.e., farms selected do not cluster in one administrative district). The final criteria ensured that farms selected were demographically reflective of the veterinary practice and varied in terms of flock and farm capacity, animal health programs, biosecurity measures, management practices, and production efficiency parameters (e.g., poorly managed to best managed flocks). As previously described (9, 31), these criteria helped ensure that the flocks enrolled were representative of most broiler chicken and turkey flocks raised in BC. Veterinarians were also instructed to distribute their sampling visits across the year to account for seasonal variations of disease pressures that may drive AMU.

Farm Surveillance Design and Laboratory Methods

Antimicrobial use, pathogen recovery and AMR data were obtained from the same flocks. A species-specific farm questionnaire (9, 31) was used to collect farm AMU and relevant production, animal health and biosecurity information. Flocks were sampled at least 1 week prior to shipment; this stage of production is closest to the consumer and also ensures that AMU until the end or last stages of the production period is captured in the questionnaire. In turkeys, all Turkey Farmers of Canada's marketing weight categories (30) were included in the sampling framework (e.g., broiler turkeys, light hens, heavy hens, light toms, heavy toms). At the time of the farm visit, pooled fecal samples were collected according to routine CIPARS farm protocol described elsewhere (9, 23, 31). In brief, 4 pooled fresh fecal samples representing the 4 quadrant of the barn were collected per flock. Each sample was cultured for *Escherichia coli, Salmonella,* and *Campylobacter*. Antimicrobial susceptibility testing was conducted using Minimum inhibitory concentrations (MIC) using an automated broth microdilution and the Clinical and Laboratory Standard Institute (CLSI) M7-A8 standards and breakpoints when available (9, 23, 31). Susceptibility of *E. coli* and *Salmonella* isolates was tested using the CMV3AGNF plate (contained 14 antimicrobials) and susceptibility of *Campylobacter* isolates was tested using the NARMS CAMPY plates (contained 9 antimicrobials) (Sensititre; Trek Diagnostic Systems, West Sussex, England) designed by the National Antimicrobial Resistance Monitoring System (NARMS) of the United States (9, 23).

Data Sources

AMU Data

Information on AMU for broiler chickens and turkeys were extracted from the CIPARS farm surveillance PostGreSQL database designed to capture the questionnaire survey data into Microsoft Excel (Office 14). The characteristics of the data collected, and detailed data collection methods are described

elsewhere (9, 23). For the AMU data used in this study, count data (farms, rations, days treated), and quantitative data (inclusion rates, milligrams of antimicrobial active ingredient and class) were extracted from the database.

AMR Data

Bacterial isolation and AMR information from flock samples were extracted from the Public Health Agency of Canada's data repository (Data Extraction and Analysis System).

Data Analysis

AMU Metrics and Indicators

AMU metrics utilized in this document were count-based (i.e., frequency of flocks), and the weight- or dose-based AMU indicators described in Equations 1–4. For the current paper, the Category IV antimicrobials (e.g., ionophores) and antimicrobials with no classification at the time of writing of this report (e.g., chemical coccidiostats, arsenicals, and pyrimethamine) were excluded.

Frequency of use (number of flocks reporting AMU/total number of flocks sampled): This count-based AMU metric was created for each poultry species and stratified by antimicrobial and route of administration.

Weight-Based Indicator. The mg/PCU was used to compare trends in AMU quantity between broiler chickens (mg/PCU_{Br}) and turkeys (mg/PCU_{Tk}) and the total poultry AMU ($mg/PCU_{poultry}$) in BC.

mg/PCU (by species): This was derived by dividing the total milligrams (mg) of antimicrobial active ingredient (AAI) administered by the biomass or PCU as per the ESVAC methodology for calculating national sales and distribution data (11). As per routine CIPARS analysis (9, 31), the PCU is calculated as the total population (minus half the cumulative mortalities recorded at the time of the farm visit) multiplied by 1 kg or 6.5 kg ESVAC standard weight at treatment for broiler chickens and turkeys, respectively. These species-specific denominators or "species PCU" described in the ESVAC's "Guidance on collection and provision of national data on antimicrobial use by animal species/categories" (12), was used to estimate the AMU quantity in broiler chickens (mg/PCU_{Br}) and turkeys (mg/PCU_{Tk}). This measure was also estimated per antimicrobial class, and for specific antimicrobials, such as TIO, GEN, and LINC-SPEC.

Equation 1. milligrams/population correction unit by species (mg/PCU_{Br}, mg/PCU_{Tk})

$$mg/PCU = \frac{AAI\ in\ feed\ (mg)\ +\ water\ (mg) + injection\ (mg)}{PCU\ (Total\ population\ \times standard\ weight\ in\ kg)}$$

$mg/PCU_{poultry}$: sum of the amount of AAI (mg) administered to broiler chickens and turkeys divided by the total poultry biomass.

Equation 2. $mg/PCU_{Poultry}$

$$mg/PCU_{poultry} = \frac{\sum mg\ AAI\ administered\ to\ broiler\ chickens\ and\ turkeys}{\sum PCU\ of\ broiler\ chickens\ and\ turkeys}$$

Dose-Based Indicators. Two dose-based indicators, nDDDvetCA/1,000 animal-days at risk and nDDDvetCA/PCU, were calculated to assess trends over time and comparability of the AMU data in broiler chickens and turkeys.

nDDDvetCA/1,000 animal-days at risk (nDDDvetCA/1,000 broiler chicken-days at risk and nDDDvetCA/1,000 turkey-days at risk)**:** This dose-based indicator was calculated by dividing the DDDvetCA (mg/kg/day) by the biomass and time-animal unit (specific days at risk for each species; this is equivalent to the age in days at pre-harvest sampling). As previously described (31), each antimicrobial was assigned a DDDvetCA following similar methodology to ESVAC's DDDvet assignment, by obtaining the average of all approved unique doses (for prevention and treatment purposes) based on Canadian drug product inserts (34, 35). The nDDDvetCA was calculated by dividing the amount of AAI used (mg) by the DDDvetCA (mg/kg/day). The DDDvetCA standards are listed in the Supplementary Materials, **Annex 1**.

Equation 3. nDDDvetCA/1,000 animal-days at risk by species (nDDDvetCA/1,000 broiler chicken-days at risk and nDDDvetCA/1,000 turkey-days at risk)

$$nDDDvetCA/_{1{,}000\ animal-days\ at\ risk} = \left(\frac{total\ antimicrobials\ (mg)/DDDvetCA_{mg/kg/day}}{total\ animals\ \times ESVAC\ std.\ weight\ (kg)\ \times days\ at\ risk}\right) \times 1{,}000$$

The average broiler chicken-days at risk used in the above calculations were 33–34 days depending on the year (as reported in the surveillance data). Average turkey-days at risk used in the above calculations were 84–89 days depending on the year (as reported in the surveillance data).

nDDDvetCA/PCU: This dose-based indicator was derived from the amount of AAI used (mg) divided by the DDDvetCA standard and the animal biomass. This was calculated for each species.

Equation 4. nDDDvetCA/PCU by species ($nDDDvetCA/PCU_{Br}$ and $nDDDvetCA/PCU_{Tk}$)

$$nDDDvetCA/PCU = \frac{\left(Total\ antimicrobials\ (mg)\ /DDDvetCA_{mg/kg/day}\right)}{\left(Total\ animal\ population\ \times ESVAC\ std.\ weight\ (kg)\right)}$$

AMR Indicators

Frequency of resistance: As per routine CIPARS AMR analysis (9, 23) at the isolate level, for *E. coli*, *Salmonella,* and *Campylobacter*, data were dichotomized into susceptible (including intermediate susceptibility) or resistant, using Clinical Laboratory Standards Institute (CLSI) breakpoints. If no CLSI interpretative criteria were available for a specific antimicrobial/bacterial combination, breakpoints were based on the distribution of MIC and harmonized with those of the United States' National Antimicrobial Resistance Monitoring System (9, 23).

Frequency of multiclass-resistance: The proportion of susceptible, resistant to 1 class and multiclass resistant isolates (resistant to 2–3 classes, resistant to 4–5 classes, and resistant

to 6–7 classes) was determined for each bacterial species as per routine CIPARS analysis (9, 23). Resistance to ≥2 antimicrobial classes is the sum of all isolates that exhibited resistance to ≥2 classes.

AMR Indicator Index (AMR Ix): This is a novel AMR indicator, calculated as the percentage of resistance (or susceptibility) to a certain antimicrobial/s, adjusted by PCU (18). The AMR Ix for poultry (AMR $Ix_{poultry}$) combines CIPARS AMR data from the broiler chickens and turkeys sampled in BC using the formula for food-producing animals described in the literature (18) and outlined in Equation 5. The organisms of interest were *Escherichia coli*, an indicator organism that is a good representative of antimicrobial exposure and the overall AMR situation (18), *Campylobacter*, a zoonotic pathogen frequently isolated from broiler chickens in Canada (36) and select organism-antimicrobial combinations specifically including those antimicrobials considered very high important and highly important to human medicine (VDD Categories I and II) (26).

Equation 5. AMR Indicator Index calculation for poultry species sampled in British Columbia

$$AMR\ Ix_{Poultry} = \frac{R_{BrY} \times .PCU_{BrY}}{PCU_{PoultryY}} + \frac{R_{TkY} \times .PCU_{TkY}}{PCU_{PoultryY}}$$

Where:

R_{BrY}-% resistance or % fully susceptible in broiler chickens (**Table 2**); calculated for all sampled flocks, per year from 2013 to 2017.

R_{TkY}-% resistance or fully susceptible in turkeys (**Table 2**); calculated per year from 2013 to 2017.

PCU_{BrY}-PCU for broiler chickens; calculated per year from 2013 to 2017.

PCU_{TkY}-PCU for turkeys; calculated per year from 2013 to 2017.

$PCU_{PoultryY}$-total PCU for all poultry species; calculated per year from 2013 to 2017.

Year-specific biomass for each species is summarized in **Table 1**.

Primary AMR Indicator Index: AMR $Ix_{Susceptible\ E.\ coli}$ was calculated as the proportion of *E. coli* isolates fully susceptible to the antimicrobials tested for by CIPARS adjusted by the PCU; this is consistent with ECDC/EFSA/EMA's primary AMR index (18).

Secondary AMR Indicator Index: Four secondary AMR Ix were determined: (1) AMR $Ix_{CRO-R\ E.coli}$, was calculated as the proportion of *E. coli* isolates resistant to ceftriaxone (CRO-R) adjusted by the PCU; this AMR Ix was used instead of the ECDC/EFSA/EMA's Extended-Spectrum Beta-Lactamases (ESBL) and AmpC- producing *E coli* since these are not yet routinely tested at CIPARS (18); (2) AMR $Ix_{\geq 2\ Multiclass-R\ E.\ coli}$ calculated as the proportion of isolates resistant to ≥2 classes of antimicrobials adjusted by the PCU; this has relevance to the monitoring of the impact of overall AMU on AMR (18); (3) AMR $Ix_{CIP-R\ Campylobacter}$, calculated as the proportion of *Campylobacter* isolates resistant to ciprofloxacin (CIP-R) adjusted by the PCU; the organism-antimicrobial combination is closely monitored by CIPARS due to the emerging resistance observed (36, 37), and; (4) AMR $Ix_{GEN-R\ E.coli}$, calculated as the proportion of isolates resistant to gentamicin (GEN-R) adjusted by the PCU. This indicator was selected as CIPARS has detected an emerging increasing trend in gentamicin use and corresponding resistance in broiler chicken isolates (31). It is important to note that, for this paper, AMR Ix for ≥2 antimicrobial classes was used instead of ECDC/EFSA/EMA's resistance to AMR Ix for ≥3 antimicrobial classes due to slight differences in isolate number (i.e., little differences between the number of isolates resistant to greater than and equal to 2 antimicrobials vs. greater than and equal to 3 antimicrobials), and CIP-R *Campylobacter* was used instead of ECDC/EFSA/EMA's CIP-R *E. coli* (i.e., there were 4 broiler chicken isolates and 1 turkey CIP-R *E. coli* isolated from the CIPARS samples between 2013 and 2017) due to more robust CIP-R *Campylobacter* data.

Integration of Poultry AMU and AMR Indicators

AMU and AMR indicators were combined into a figure to descriptively assess potential similarities in trends over time: (1) AMU frequency and AMR (% R), by species, for use of, and *E. coli* resistance to, CRO, GEN, and LINC-SPEC and (2) AMU in mg/PCU and AMR Ix for the following: (a) total AMU for broilers and turkeys across all antimicrobials (mg/PCU) and AMR $Ix_{Susceptible\ E.\ coli}$ and AMR $Ix_{\geq 2\ Multiclass-R\ E.\ coli,}$ (b) TIO mg/PCU and AMR $Ix_{CRO-R\ E.coli,}$ and (c) GEN and LINC-SPEC $mg/PCU_{poultry}$ and AMR $Ix_{GEN-R\ E.coli.}$

Descriptive and Temporal Analysis

Analysis of the data was conducted in Microsoft Excel (Office 14), Stata SE Version 15 (College Station, Texas) and SASv12.1 (Cary, North Carolina).

AMU. Temporal changes were determined following routine CIPARS analysis protocols (9, 23). In brief, frequency of AMU by AAI during the most recent surveillance year (2017 referent year) was compared to the initial surveillance year (2013), and the preceding year (2016) using logistic regression models (asymptotic or exact models depending on prevalence of the outcome variable). Models were developed with year as a categorical independent variable and using $P \leq 0.05$ for significance (i.e., marked by the use of the words "significant" or "significantly" throughout the text). For the AMU indicators, data by antimicrobial class and the total of all classes per year were described. Changes in AMU indicators, between surveillance years were expressed as percent change (i.e., current year or initial surveillance year minus previous year divided by the previous year or initial surveillance year then multiplied by 100).

AMR. Resistance prevalence estimates were adjusted for clustering at the flock level using generalized estimating equations (GEE) with a binary outcome, logit-link function, and exchangeable correlation structure. Null binomial response models were run for each antimicrobial and from each null model, the intercept (β_0) and 95% confidence intervals were used to calculate population-averaged prevalence estimates using the formula $[1 + \exp(-\beta_0)]^{-1}$. For the temporal analysis,

TABLE 1 | Farm characteristics of CIPARS broiler chicken and turkey layer flocks sampled in British Columbia.

	2013	2014	2015	2016	2017	Total	Mean	Std. dev.
BROILER CHICKENS								
Number of flocks sampled, *n*	**26**	**30**	**25**	**32**	**30**	**143**	29	3
Population (number of birds)	522,525	650,756	592,652	765,987	732,417	3,264,336	652,867	99,679
Biomass (using 1 kg ESVAC weight)	522,525	650,756	592,652	765,987	732,417	3,264,336	652,867	99,679
Days at risk (average)	33	33	33	33	34	N/A	33	0
Milligrams active ingredients	54,512,352	67,656,030	54,790,215	73,658,806	71,972,475	322,589,877	64,517,976	9,269,757
mg/PCU	104	104	92	96	98	99	99	5
TURKEYS								
Number of flocks sampled, *n*	**29**	**29**	**30**	**30**	**27**	**145**	29	1
Population (number of birds)	253,930	270,750	267,228	303,641	246,046	1,341,594	268,319	22,124
Biomass (using 6.5 kg ESVAC weight)	1,650,542	1,759,872	1,736,982	1,973,663	1,599,299	8,720,358	1,744,072	143,805
Days at risk	87	84	88	88	89	N/A	87	2
Milligrams active ingredients	149,355,383	120,425,553	74,654,795	219,925,956	225,819,340	790,181,027	158,036,205	64,937,031
mg/PCU	90	68	43	111	141	91	91	38
BROILER CHICKENS AND TURKEYS								
Population, total	776,455	921,506	859,880	1,069,627	978,463	4,605,930	921,186	111,827
Biomass (PCU), total	2,173,067	2,410,628	2,329,634	2,739,650	2,331,716	11,984,694	2,396,939	210,084
Milligrams active ingredients, total	203,867,735	188,081,583	129,445,009	293,584,762	297,791,815	1,112,770,904	222,554,181	72,306,477
mg/PCU, total	94	78	56	107	128	93	93	27

ESVAC, European Surveillance for Veterinary Antimicrobial Consumption; PCU, population correction unit.

models were developed similar to those described above for the analysis of AMU data with year as a categorical independent variable and $P \leq 0.05$ for significance. Temporal changes in multiclass resistance prevalence are expressed as percent change in multiclass resistance (i.e., current year or initial surveillance year minus previous year divided by the previous year or initial surveillance year then multiplied by 100).

AMR Ix. Change in AMR Ix, by indicators, between surveillance years are expressed as percent change (i.e., current year or initial surveillance year minus previous year divided by the previous year or initial surveillance year then multiplied by 100).

RESULTS

Farm Characteristics

Farm-level characteristics during the study period are summarized in **Table 1**. From 2013 to 2017, a total of 143 broiler flocks (Mean: 29 flocks/year) and 145 turkey flocks (Mean: 29 flocks/year) were sampled. The total biomass (i.e., estimated based on ESVAC standard weight at treatment) was 3.6 million kg broiler chickens (Mean: 0.65 million kg/year) and 8.72 million kg turkeys (Mean: 1.7 million kg/year). The broiler chicken and turkey flocks were sampled by all the major veterinary practices in BC and the poults/chicks sampled originated from all the major hatcheries located in the province. Overall, 25% of flocks were classified as RWA or were a part of an antibiotic-free program (ABF) and organic. These flocks were not using medicated feed, including ionophores and chemical coccidiostats, from the time of chick or poult placement to the time of pre-harvest sampling.

AMU Metrics and Indicators

Count Based AMU Metric: Frequency of Use

Table 2 summarizes AMU frequency by route of administration and by VDD categorization of antimicrobials (26). In broilers, the most frequently used antimicrobials in feed were bacitracin (BAC) (46%), virginiamycin (VIR) (37%), and penicillin G procaine (PEN) (28%). The frequency of VIR use decreased significantly from 54% in 2013 to 23% in 2017 ($P \leq 0.05$). Avilamycin (AVI) was used beginning in 2014 and the frequency of use increased from 7 to 23%. The frequency of flocks not reporting any AMU in feed increased significantly, from 13% (2013) to 37% (2017) ($P \leq 0.05$). In turkeys, the top 3 ranking antimicrobials had similar frequency to broilers: BAC (51%), VIR (40%), and PEN (5%).

Antimicrobials administered via water were infrequently used both in broiler chickens (1–3% overall) and turkeys (1–4%) (**Table 2**). Two broiler flocks (2013) and one turkey flock (2017) reported use of enrofloxacin (ENR), a fluoroquinolone which is not labeled for use in poultry in Canada (deemed extra-label use if administered in species than cattle, pigs, dogs and cats and administered in routes other than injection).

As for the injectable antimicrobials, the reported frequency of ceftiofur (TIO) use in broiler chickens decreased from 58% (2013) to 7% in 2014, with none reported from 2015 to 2017. The use of GEN was consistently reported during the study timeframe; the frequency peaked in 2015 (40%), decreased to 6% in 2016, and then increased to 17% in 2017. Lincomycin-spectinomycin was reportedly used for the first time in broilers in 2015 (20%), then in 2016 use dropped to 3%, and in 2017 it increased to 7%. Flocks not using antimicrobials at the hatchery level varied depending on the year (dropped to 40%

TABLE 2 | Antimicrobial use frequency (number of flocks reporting use/total number of flocks sampled) in CIPARS broiler chicken and turkey flocks in British Columbia, 2013-2017.

Years		2013	2014	2015	2016	2017	All years
BROILERS							
Number of flocks		26	30	25	32	30	143
Feed							
II	Penicillin G potassium	0%	17%	20%	0%	0%	7%
	Penicillin G procaine	50%	7%	24%	31%	30%	28%
	Virginiamycin	**54%[a]**	34%	36%	41%	**23%[a]↓**	37%
III	Bacitracin	50%	45%	36%	50%	50%	46%
NA	Avilamycin	0%	7%	12%	16%	23%	12%
No antimicrobials used in feed		**13%[a]**	34%	24%	25%	**37%[a]↑**	27%
Water							
I	Enrofloxacin	8%	0%	0%	0%	0%	1%
II	Amoxicillin	0%	0%	4%	0%	3%	1%
	Penicillin	4%	3%	0%	0%	0%	1%
	Penicillin-streptomycin	0%	0%	4%	3%	7%	3%
III	Sulfaquinoxaline	0%	3%	0%	3%	0%	1%
	Tetracycline	0%	0%	0%	0%	3%	1%
	Tetracycline-neomycin	0%	0%	0%	0%	3%	1%
	No antimicrobials used in water	88%	93%	92%	94%	90%	91%
Injections							
I	Ceftiofur	58%	7%	0%	0%	0%	12%
II	Gentamicin	12%	20%	40%	6%	17%	18%
	Lincomycin-spectinomycin	0%	0%	20%	3%	7%	6%
	No antimicrobials used at the hatchery	35%[a]	73%	40%	91%	77%[a↑]	65%
TURKEYS							
Number of flocks		**29**	**29**	**30**	**30**	**27**	**145**
Feed							
II	Tylosin	0%	0%	0%	0%	7%	1%
	Penicillin G potassium	0%	0%	3%	0%	0%	1%
	Penicillin G procaine	0%	21%	0%	3%	0%	5%
	Virginiamycin	**17%[a]**	38%	67%	33%	**44%[a]↑**	40%
	Trimethoprim-sulfadiazine	0%	0%	0%	0%	4%	1%
III	Bacitracin	69%	55%	23%	57%	52%	51%
	Chlortetracycline	3%	3%	0%	0%	4%	2%
	No antimicrobials used in feed	24%	10%	17%	13%	11%	15%
Water							
I	Enrofloxacin	0%	0%	0%	0%	4%	1%
II	Neomycin	3%	3%	0%	3%	0%	2%
	Penicillin G procaine	3%	0%	3%	10%	4%	4%
	Penicillin-streptomycin	0%	0%	0%	10%	4%	3%
III	Oxytetracycline-neomycin	0%	0%	0%	0%	4%	1%
	Tetracycline	3%	3%	0%	0%	0%	1%
	Tetracycline-neomycin	3%	3%	0%	0%	0%	1%
	No antimicrobials used in water	90%	93%	97%	87%	93%	92%
I	Ceftiofur	3%	0%	0%	0%	0%	1%
II	Gentamicin	76%	90%	73%	83%	81%	81%
	No antimicrobials used at the hatchery	21%	10%	27%	17%	19%	19%

Roman numerals I to IV indicated categories of importance to human medicine as outlined by the Veterinary Drugs Directorate, Health Canada; N/A not applicable (no classification at the time of writing of this manuscript). Antimicrobials included in the Table are routinely reported by countries to the OIE and include those with GP properties. Please note that there were 3 broiler chicken locks with no feed/water information. Numbers may not add up to 100% as some flocks were treated with multiple antimicrobials during the grow-out cycle (feed, water, injection). [a]*significant ($P \leq 0.05$) difference between 2013 and 2017, highlighted in bold fonts. The arrows indicate the direction of the shift (increased or decreased).*

in 2015) but between 2013 and 2017, the number of flocks significantly increased from 35 to 77% ($P \leq 0.05$). In turkeys, there was only one flock (3%) with reported use of TIO in 2013, with no use reported from 2014 to 2017. Gentamicin was consistently used in turkey poults (73–90%). Turkey flocks not using any antimicrobials at the hatchery were generally stable over time (17–27%).

Weight- and Dose-Based AMU Indicators

Figure 1 summarizes the AMU temporal trends in broiler chickens and turkeys, using different weight- and dose-based indicators. Data can be found in **Annex 2**.

Weight-based (mg/PCU): In broilers, the mg/PCU_{Br} decreased by 6% between 2013 and 2017 (104–98 mg/PCU_{Br}) and during the last 2 years, it increased marginally by 2% (96–98 mg/PCU_{Br}). In turkeys, the mg/PCU_{Tk} decreased by 13% between 2013 and 2017 (90–78 mg/PCU_{Tk}), but during the last 2 years, 2016–2017, it increased significantly by 61% (49–78 mg/PCU). There was a decrease in mg/PCU_{Tk} between 2014 and 2015 due to the decrease in BAC use (49–17%) reported in the sampled turkey flocks. The magnitude of change between the antimicrobial classes varied depending on the year or antimicrobial class.

Dose-based (number of DDDvetCA/1,000 animal-days at risk): In broilers, nDDDvetCA/1,000 broiler-chicken-days at risk decreased by 11% between 2013 and 2017 (484–431 nDDDvetCA/1,000 broiler chicken days at risk), with a 13%

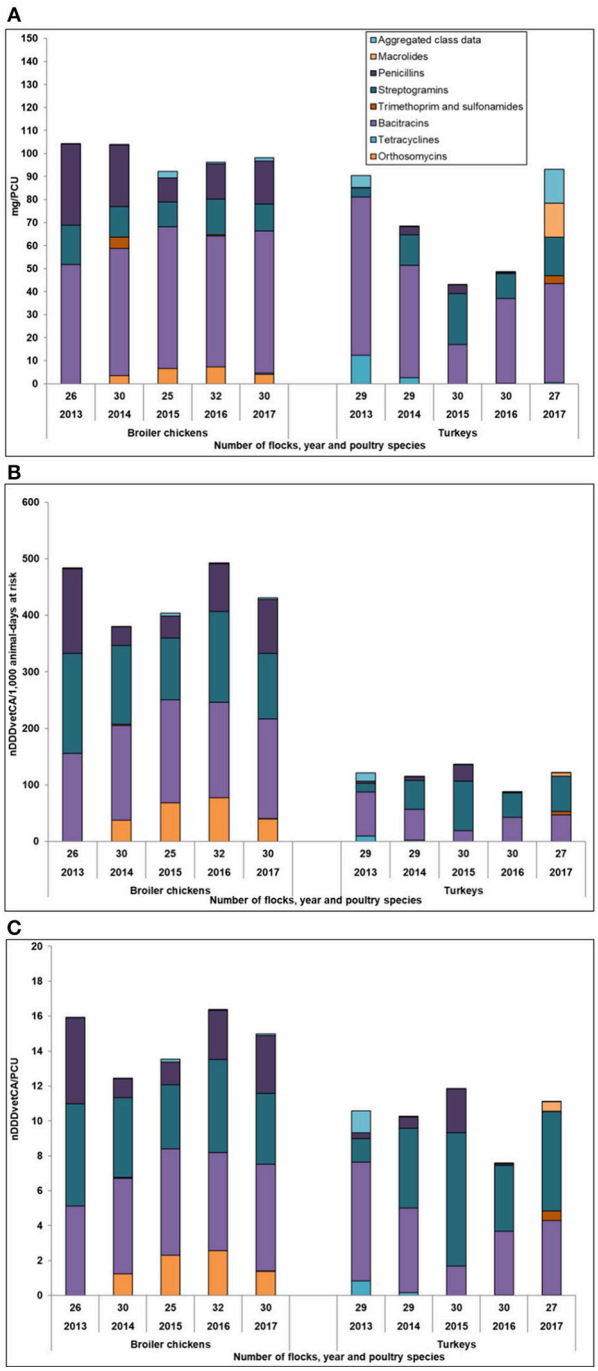

FIGURE 1 | Antimicrobial use indicators comparing broiler chickens and turkeys, 2013–2017. **(A)** Milligrams/population correction unit. **(B)** Number of defined daily doses in animals/1,000 animal-days at risk. **(C)** Number of defined daily doses in animals/population correction unit. Aggregated class data was comprised of 3rd generation cephalosporins, fluoroquinolones, aminoglycosides, and lincosamides-aminocyclitols.

(493–431) decrease from 2016 to 2017. As with the mg/PCU_{Br}, AVI (orthosomycins) gradually increased between 2014 and 2016 but decreased in 2017. In turkeys, this AMU indicator fluctuated over time due to the shifts in the use of 3 antimicrobial classes, BAC, VIR (streptogramins), and PEN. Unlike in broiler chickens, overall, it decreased by 11% between 2013 and 2017, but remarkably increased between 2016 and 2017 by 40% (88–122 nDDDvetCA/1,000 turkey-days at risk). The increase during the last 2 years was primarily due to the increase in the use of BAC and VIR and TMPS (trimethoprim-sulfonamide) (reported for the first time in BC in 2017). Compared to broiler chickens, the yearly total values for turkeys were lower.

Dose-based (number of DDDvetCA/PCU): Between 2016 and 2017, the nDDDvetCA/PCU_{Br} decreased by 9% while the nDDDvetCA/PCU_{Tk} increased by 46%, but during the study timeframe (2013–2017), this indicator showed a marginal change in the total nDDvetCA/PCU [nDDvetCA/PCU_{Br}: 16 to 15 (6%); nDDDvetCA/PCU_{Tk}: 10.6 to 11.1 (5%)].

Figure 1 and **Annex 2** summarizes the trends in the AMU indicators illustrating the inconsistencies of the temporal patterns across the 3 AMU indicators within a poultry species. In broiler chickens, mg/PCU_{Br}, nDDDvetCA/1,000 broiler chicken-days at risk, and the nDDDvet/PCU_{Br} decreased between 2013 and 2017. However, during the later 2 years of the study timeframe, the mg/PCU_{Br} increased while the 2 dose-based indicators decreased. This was due to the shifts in the use of certain antimicrobial classes such as increased penicillins and bacitracins [DDDvetCA standards at 10.1 and 5.4, respectively (**Annex 1**)] and decreased streptogramins and orthosomycins (DDDvetCA standard of 2.9 for both) uses. In turkeys, overall, between 2013 and 2017, the mg/PCU_{Tk} decreased while the dose-based indicators increased. Between 2016 and 2017, the 3 indicators notably increased due to the shift in the use of streptogramins and bacitracins, and of macrolides (DDDvetCA standard of 26) and trimethoprim-sulfonamides (DDDvetCA standard of 10.5 and 2.2.); the latter 2 antimicrobial uses occurred in BC for the first time in 2017.

AMR Indicators

Frequency of resistance (%R): The frequency of resistance to select antimicrobials in *E. coli*, *Salmonella,* and *Campylobacter* from the broiler chickens and turkeys are presented in **Figure 2** and significant temporal changes are summarized in **Annex 3**. The frequency of resistance for each antimicrobial-organism combination varied by poultry species. Over time, CRO-R decreased while GEN-R increased in *E. coli* and *Salmonella* isolates and CIP-R increased in *Campylobacter* from broiler chickens and turkeys.

Broiler chickens–E. coli: From 2013 to 2017, resistance to ceftriaxone (CRO-R) decreased from 63 to 21%, while resistance to gentamicin (GEN-R) increased from 8 to 21%. Resistance to nalidixic acid (NAL-R) was detected in 9–19% of the isolates. Streptomycin resistance and TET-R increased significantly between 2013 and 2017.

Broiler chickens–Salmonella: Resistance to CRO was 18% in 2013, it decreased from 32% in 2015 (highest observed) to 10% in 2017. Nalidixic acid resistance was detected in 2013 (5%) and 2015 (30%) but no NAL-R *Salmonella* isolates were recovered in 2016 or 2017. Gentamicin resistance emerged between 2014 and 2017 (1 to 4%).

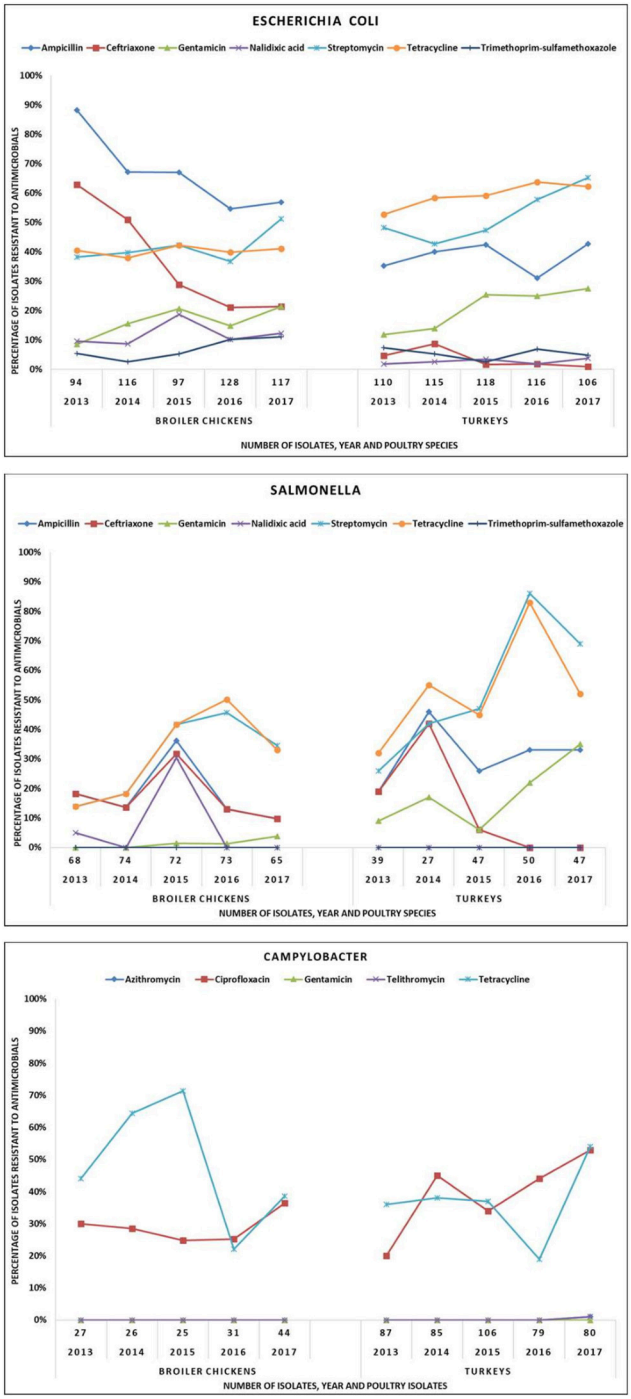

FIGURE 2 | Temporal variations of resistance in *Escherichia coli, Salmonella,* and *Campylobacter* in broiler chickens and turkeys, 2013–2017.

Broiler chickens–Campylobacter: Ciprofloxacin resistance significantly ($P \leq 0.05$) increased between 2013 (30%) and 2017 (36%).

Turkeys–E. coli: Ceftriaxone resistance was generally lower than in broilers (9% peak in 2014) and remained stable (1–2%) between 2015 and 2017. Gentamicin resistance significantly ($P \leq 0.05$) increased from 12% (2013) to 27% (2017).

Turkeys–Salmonella: Ceftriaxone resistance peaked at 42% in 2014 then decreased to 6% in 2015 and no resistant isolates were detected in 2016 and 2017. Gentamicin resistance fluctuated over time, but markedly increased between 2015 and 2017 (6–35%).

Turkeys–Campylobacter: Ciprofloxacin resistance significantly increased ($P \leq 0.05$) from 20% in 2013 to 53% in 2017. Tetracycline resistance increased significantly ($P \leq 0.05$) from 36% in 2013 to 54% in 2017; compared to 2016, TET-R increased significantly ($P \leq 0.05$) from 19 to 54%.

Multiclass Resistance

Figure 3 demonstrates the multiclass resistance patterns of *E. coli*, *Salmonella,* and *Campylobacter* isolated from the 2 poultry species. Overall there were 4 *E. coli* isolates (3 from broiler chickens and 1 from turkeys) that were resistant to 6–7 antimicrobial classes detected during the study timeframe. None of the *Campylobacter* isolates were resistant to 4 or more classes of antimicrobials. The distribution of susceptible and multiclass resistant isolates varied over time across the 2 poultry species (data are presented in **Annex 5**). In broiler chickens, fully susceptible *E. coli* generally increased over time (9–21%), in contrast to the decreasing trend observed amongst the turkey isolates (35–21%). As for *Salmonella,* fully susceptible isolates decreased in both broiler chickens (2013: 79%, 2017: 55%) and turkeys (2013: 56%, 2017: 19%). Amongst *Campylobacter*, an increase in fully susceptible isolates was noted in broiler chickens (41–52%) while a decrease was noted in turkeys (62–29%).

AMR Index

Results are summarized in **Table 3**.

Primary AMR indicator: The AMR Ix $_{\text{Susceptible } E.coli}$ decreased by 34% from 0.29 in 2013 to 0.19 in 2017.

Secondary AMR indicator: The AMR Ix $_{\text{CRO}-\text{R } E. coli}$ decreased by 61% from 0.19 in 2013 to 0.07 in 2017. During the same timeframe, the AMR Ix $_{\geq 2\text{Multiclass}-\text{R } E. coli}$ increased by 12% from 0.53 in 2013 to 0.59 in 2017; AMR Ix $_{\text{CIP}-\text{R } Campylobacter}$ increased by 79% from 0.23 to 0.41, AMR Ix $_{\text{GEN}-\text{R } E.coli}$ markedly increased by 103% from 0.11 in 2013 to 0.22% in 2017.

Integration of AMU (Frequency) and AMR Data

The integration of the percentage (with low and high confidence limits) of CRO-R *E. coli* (**Figure 2** and **Annexes 3, 4**) and the percentage of flocks that reported TIO use (**Table 2**) is depicted in **Figure 4A**. The use of TIO at the broiler hatcheries corresponded with the high CRO-R in 2013; both CRO-R and TIO use dropped in 2014, but despite no reported AMU in 2015–2017, CRO-R persisted at low level. There were few CRO-R isolates detected in turkeys (5%), which corresponded with a low TIO use (1 flock) in 2013. The relationship between the percentage of flocks that reported GEN and LINC-SPEC use and the percentage of GEN-R *E. coli* isolates is depicted in **Figure 4B**. The use of GEN and

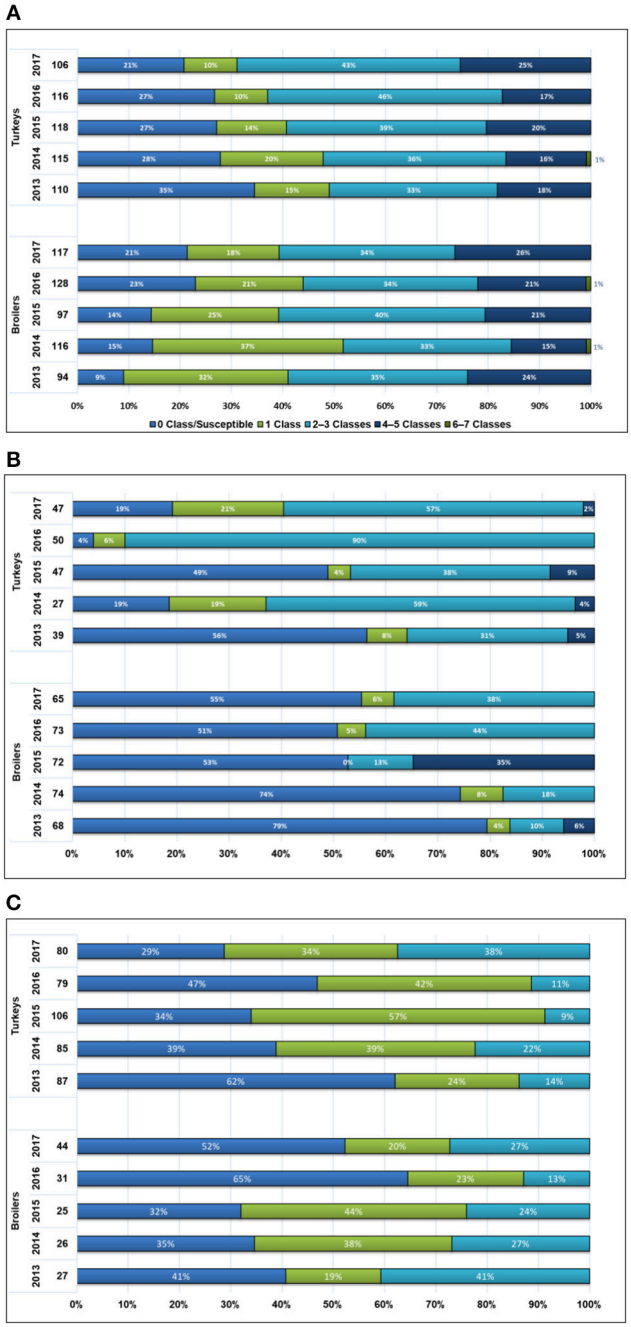

FIGURE 3 | Multiclass resistance data in broiler chickens and turkeys, 2013–2017. **(A)** *Escherichia coli*, % of antimicrobial classes in resistance patterns. **(B)** *Salmonella*, % antimicrobial classes in resistance patterns. **(C)** *Campylobacter*, % antimicrobial classes in resistance pattern. As per routine CIPARS analysis (9, 23), the number of isolates by antimicrobial classes in the resistance pattern were grouped into 5 resistance patterns as follows: 0, 1, 2–3, 4–5, and 6–7.

LINC-SPEC in broiler chicks at the hatchery corresponded with the increase in GEN-R over time. The same trend was noted in turkeys, but unlike in broiler chickens, GEN was the only antimicrobial used.

TABLE 3 | Antimicrobial use and resistance indicator summary, CIPARS broiler chicken and turkey flocks in British Columbia, 2013–2017.

	2013	2014	2015	2016	2017
PRIMARY AMU INDICATOR[a]					
$mg/PCU_{poultry}$, total AMU	94	78	56	107	128
$mg/PCU_{poultry}$, ceftiofur use	0.08	0.01	0.00	0.00	0.00
SECONDARY AMU INDICATOR[a]					
$mg/PCU_{poultry}$, gentamicin and lincomycin-spectinomycin use	0.04	0.06	0.28	0.03	0.07
PRIMARY AMR INDICATOR[a]					
AMR $Ix_{Susceptible\ E.\ coli}$	0.29	0.24	0.24	0.26	0.19
SECONDARY AMR INDICATOR[b]					
AMR $Ix_{CRO-R\ E.\ coli}$	0.19	0.20	0.09	0.07	0.07
AMR $Ix_{\geq 2Multiclass-R\ E.\ coli}$	0.53	0.50	0.60	0.61	0.59
AMR $Ix_{CIP-R\ Campylobacter}$	0.23	0.40	0.35	0.42	0.41
AMR $Ix_{GEN-R\ E.\ coli}$	0.11	0.14	0.24	0.22	0.22

AMU, antimicrobial use; AMR, antimicrobial resistance; mg/PCU, milligrams/population correction unit; $mg/PCU_{poultry}$, the total mg/PCU in broiler chickens and turkeys combined; AMR Ix, antimicrobial resistance indicator index; CRO-R, ceftriaxone-resistant; CIP-R, ciprofloxacin-resistant; GEN-R, gentamicin-resistant; ≥2 Multiclass-R, sum of all isolates that exhibited resistance to 2 or more classes of antimicrobials; [a,b]Please refer to the methods for the description of these indicators.

Ciprofloxacin-resistant *Campylobacter* trends for both broiler chickens and turkeys generally increased but no corresponding increase in reported ENR use was noted during the study timeframe as the use of this antimicrobial, a veterinary fluoroquinolone not approved for use in any poultry species in Canada, was relatively low (2 broiler flocks and 1 turkey flock, **Table 2**).

Integration of AMU in mg/PCU and AMR Index (AMR Ix)

The findings are summarized in **Table 3** and the relationship between AMU and AMR Ix is demonstrated in **Figure 5**. During the study timeframe (2013–2017), the total mg/PCU for broiler chickens and turkeys increased (94–128) which corresponded to the decrease in AMR Ix $_{Susceptible\ E.\ coli}$ (0.29–0.19, decreased by 34%). However, AMR Ix $_{\geq 2Multiclass-R\ E.\ coli}$ paralleled the trends in total mg/PCU between 2013 and 2016 (increased) but dropped by 10% between 2016 (0.61) and 2017 (0.59). **Figure 5** also depicts the relationship between the TIO use in $mg/PCU_{poultry}$ and the AMR Ix $_{CRO-R\ E.coli.}$ The TIO $mg/PCU_{poultry}$ decreased from 0.08 in 2013 to 0.01 in 2014, and no TIO use was reported from 2015 to 2017. A corresponding decrease in AMR Ix $_{CRO-R E.coli}$ was noted over time as previously described (**Table 3**) however, it is important to note that despite no reported TIO use since 2014, the AMR Ix $_{CRO-R\ E.coli}$ persisted until 2017. Also in **Figure 5**, the highest reported GEN and LINC-SPEC use was recorded in 2015 at 0.28 mg/PCU and decreased in 2016 (0.03 $mg/PCU_{poultry}$) to 2017 (0.07 $mg/PCU_{poultry}$) (**Table 3**) but there was no corresponding decrease in AMR Ix $_{GEN-R\ E.coli}$ from 0.24 in 2015 (peak of use) to 0.22 in 2016 and 2017.

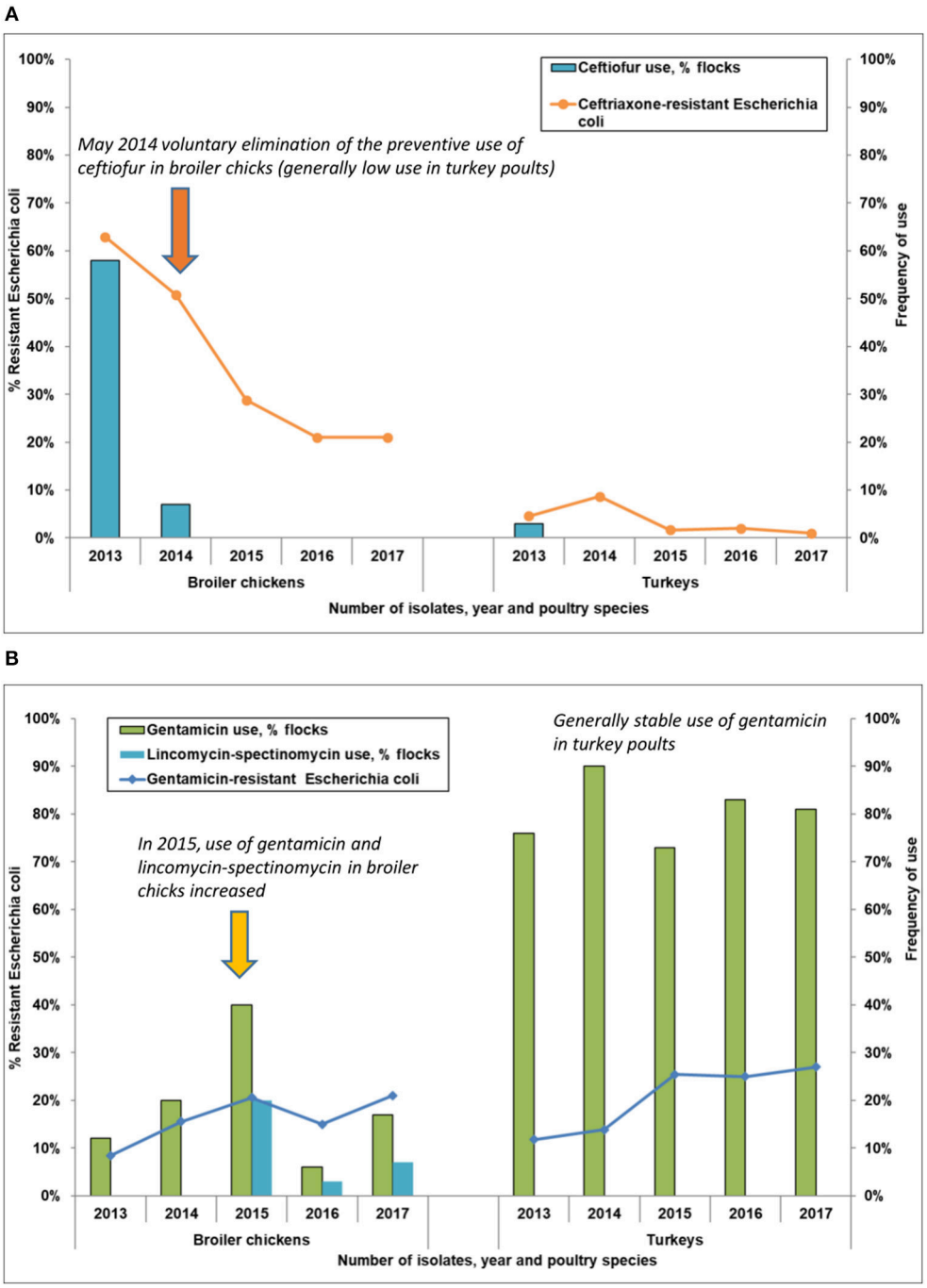

FIGURE 4 | Antimicrobial use frequency and antimicrobial resistance in *Escherichia coli* in broiler chickens and turkeys. **(A)** Percentage of flocks using ceftiofur and percentage of *Escherichia coli* isolates resistant to ceftriaxone. **(B)** Percentage of flocks using gentamicin and lincomycin-spectinomycin and percentage of *Escherichia coli* isolates resistant to gentamicin.

DISCUSSION

This paper described temporal trends in AMU and AMR in poultry flocks, compared AMU and AMR between the poultry species, and evaluated the effects of various AMU and AMR units of measurement for reporting flock-level data on AMU and AMR. The farm-level data provided a comprehensive overview of AMU in the broiler chicken and turkey sectors within the province in Canada. Our data showed that the direction and magnitude of either trends or discrepancies between populations could change based on the metric or indicator chosen, but when the indicators were applied simultaneously to the same dataset,

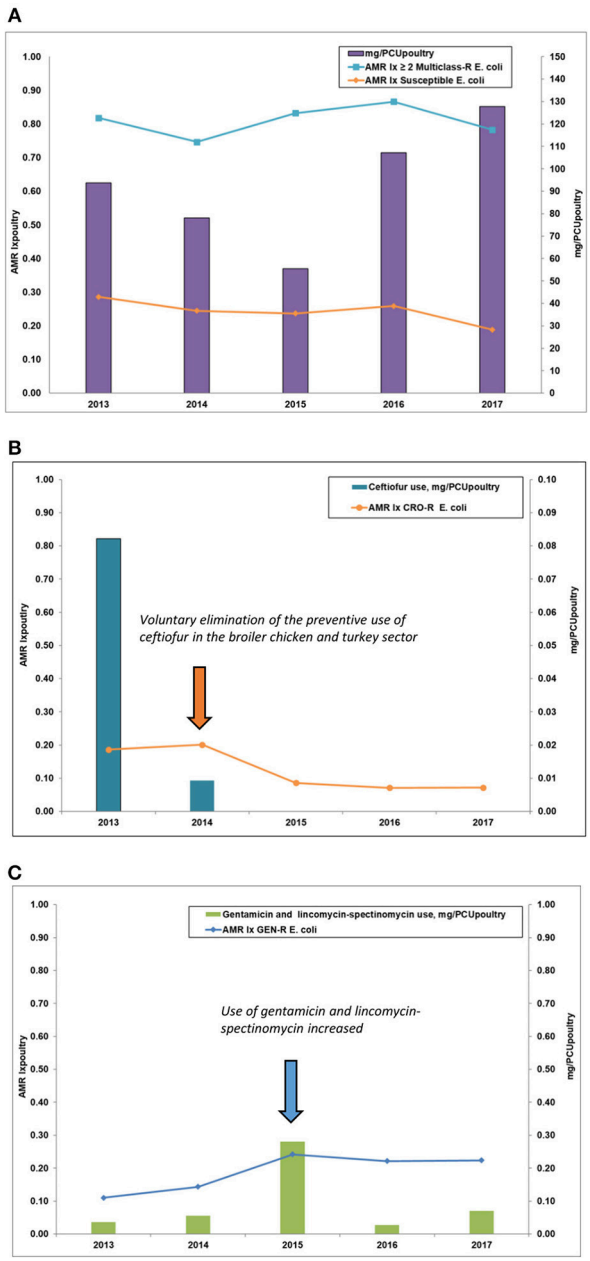

FIGURE 5 | Milligrams/population correction unit (mg/PCU) and antimicrobial resistance indicator index (AMR Ix) for *Escherichia coli* in broiler chickens and turkeys. **(A)** Milligram/population correction unit$_{poultry}$ (total use) and AMR Ix (multiclass/susceptible isolates). **(B)** Milligram/population correction unit$_{poultry}$ (ceftiofur use) and AMR Ix (ceftriaxone resistance). **(C)** Milligram/population correction unit$_{poultry}$ (gentamicin and lincomycin-spectinomycin use) and AMR (gentamicin resistance). AMR, antimicrobial resistance; mg/PCU$_{poultry}$, the total milligrams/population correction unit in broiler chickens and turkeys combined; AMR Ix, antimicrobial resistance indicator index; CRO-R, ceftriaxone-resistant; GEN-R, gentamicin-resistant, ≥2 Multiclass-R, sum of all isolates that exhibited resistance to 2 or more classes of antimicrobials.

multiple study objective/s described above could be achieved. Hence, we believe there is much value to a comprehensive reporting of AMU data using different indicators.

The count-based measurement (frequency of flocks medicated), when used by itself in a surveillance program, can detect changes over time; indicate how extensively on antimicrobial is used; detect shifts in AMU, particularly the shifts in the use of antimicrobials with higher importance to human medicine to antimicrobials with lower importance to human medicine or vice versa; and highlight the proportion of farms not using antimicrobials, which can be an indication of the changing production profile (i.e., increase in the number of farms raising ABF/RWA or organic birds). In Canada, frequency data have been used in studies and risk assessments exploring the link between AMU and AMR, for example, the use of TIO in poultry and AMR among *Salmonella* Heidelberg and *Escherichia coli* (38).

The weight-based indicator, mg/PCU, is becoming one of the most frequently used AMU indicators internationally (39). In the Canadian poultry industry, total population and farm-level efficiency data including daily and cumulative mortalities can be accessed from farm records as per the on-farm food safety program requirements (34, 35), feed mill delivery receipts and prescription data for medicated feed. As such, this indicator makes use of farm data already collected. The utility of this weight-based indicator for monitoring AMU during the early implementation phase of a national AMU surveillance program has been cited in the literature to quantify overall national level use (39, 40). In Europe, this indicator consistently showed a statistically significant negative association between AMU and susceptible isolates, and thus was cited as "the most adequate indicator" to monitor the impact of AMU reduction (17, 18).

The dose-based indicator, nDDDvetCA adjusted by animal time (1,000 animal-days at risk), or biomass (PCU) is currently used by 8/16 countries participating in the AACTING network. It was also cited for animal AMU reporting in the European Union/European Economic Area (12). The DDDvet is a technical unit of measurement that, unlike other indicators, account for dosing differences between active ingredients, formulations, and animal species (41).

All three AMU indicators used; mg/PCU, nDDDvetCA/1,000 animal-days at risk, and nDDDvetCA/PCU detected temporal AMU changes (i.e., total AMU and by class). The reduction in total AMU (mg/PCU) could indicate early industry actions in anticipation of the forthcoming changes in industry policies (24, 25). The magnitude of change over time varied depending on the antimicrobial class, poultry species and year. The increase noted in the dose-based indicators, specifically from 2016 to 2017, is an indication of the changes in antimicrobial preferences (i.e., switch from antimicrobials with higher daily doses to those with lower daily doses), illustrating the value of this indicator over non-dose-based indicators. As we described previously (31), the DDDvetCA assignment (**Annex 1**) involved stratification by route of administration (e.g., feed, water, and injection); this methodology was modified from ESVAC DDDvet assignment principles (41), where only one DDDvetCA was assigned for any oral formulation. This greater stratification of DDDvetCA assignment could be used for detecting shifts in use from one route of administration to another, such as potential shift from feed uses (i.e., preventive doses, prolonged days of

administration) to water uses (i.e., treatment doses and shorter days of administration). With the anticipated changes in disease prevalence (e.g., necrotic enteritis) in the broiler chicken and turkey sectors following the implementation of the second and third steps of the industry AMU strategy (i.e., elimination of the preventive use of VDD Category II and III antimicrobials which are largely used for managing necrotic enteritis in the field), this indicator will permit detection of the shift in AMU as described above.

The three indicators enabled inter-species AMU comparison. In Europe, the mg/PCU indicator is deemed the primary AMU indicator for national sales and distribution data to monitor overall impact of AMU policy change due to its ability to detect changes in AMU over time and robustness in describing the animal population exposed (18). With the use of mg/PCU, mg/PCU_{Tk} AMU was generally lower compared to mg/PCU_{Br}. The practice of feeding unmedicated rations during the late growing to finishing stages of the production period in turkeys is potentially driving the lower AMU. When nDDDvetCA/1,000 animal-days at risk was used, the magnitude of the difference between turkeys and broiler chickens was greater than the mg/PCU. This may be due to the relatively larger number of days required to achieve marketing weight for some categories, such as heavy tom turkeys. The turkey and broiler chicken sectors of the Canadian poultry industry collectively agreed to eliminate the preventive use of VDD Category II antimicrobials by the end of 2018 and VDD Category III antimicrobials by the end of 2019 (25). Additionally, by the end of 2018, the use of VDD Categories I to III antimicrobials requires veterinary oversight or to be used only with a veterinary prescription (28). It is therefore expected that the quantities reported via the surveillance data will decrease over time across all three indicators.

For AMR reporting, the percentage of isolates resistant (% R) to a specific antimicrobial is a standardized unit of measurement used internationally for reporting resistance prevalence from animals and humans. This complements trends in AMU data to assess the impact of an AMU reduction strategy (42, 43).

The AMR Ix is a new indicator that combines the frequency of resistance across the host species under surveillance, while accounting for the relative contribution of each of the host species on the overall AMR Ix (18). In this study, the antimicrobials selected for AMR Ix estimation for *E. coli* were based on their relevance to overall AMU selection pressure and their importance to human medicine (18). A zoonotic pathogen, *Campylobacter,* was included to monitor the temporal changes in CIP-R; one of the emerging antimicrobial-resistant strains of high interest in Canada that is closely monitored by CIPARS (9, 36, 37). *Salmonella* is another food-borne pathogen in Canada (44), but because of serovar variations in resistance among *Salmonella* and the unique spectrum of serovars detected by species, we did not estimate an AMR Ix for this zoonotic pathogen. Reduction targets for AMR in Canada have not been established; however, the AMR Ix could be used to monitor AMU reduction strategies, and potentially for monitoring the progress of the implementation of the industry interventions to reduce AMR.

In this study, the AMR Ix indicated success of an industry intervention and simultaneously, the unintended consequence of the intervention (i.e., AMR Ix $_{CRO-R\ Ecoli}$ decreasing trend and AMR Ix $_{GEN-R\ Ecoli}$ increasing trend, indicative of the shift for treating infections in young chicks from TIO to GEN). The AMR Ix is also a good complement to mg/PCU in monitoring the overall impact of changes in antimicrobial usage patterns as demonstrated in the integrated figures, such as the parallel trends noted between $mg/PCU_{poultry}$ and the AMR $Ix_{\geq 2Multiclass-R\ E.coli}$ or the disparate trends observed between $mg/PCU_{poultry}$ and AMR Ix $_{Susceptible\ E.\ coli.}$ The latter observation is consistent with the literature (i.e., a consistent statistically significant association between total AMU and susceptible isolates) (18). Further analysis of the relationship between these trends in AMU and AMR indicators is required in order to investigate their statistical significance when additional risk factors for AMR are considered. When more robust data from the CIPARS program becomes available (e.g., ongoing data collection from all relevant livestock and poultry species), associations among indicators could be further assessed to substantiate the findings presented in this paper.

Surveillance data indicated that 8% of broiler flocks in 2013 and 4% of turkey flocks in 2017 reported fluoroquinolone use, with no use from 2014 to 2016. In BC in 2014, ∼38 kg of fluoroquinolones were distributed by BC livestock and poultry veterinarians (45), however, the actual kg of fluoroquinolones distributed and used in poultry production is unknown. The frequency of CIP-R in *Campylobacte*r remained moderately high and the reason for the persistence in Canadian flocks is unclear and ongoing monitoring and research is needed to determine the main drivers of CIP-R in poultry. The literature indicates that mutation in the Thr-86-Ile mutation in gyrA is directly assocaited with the enhanced fitness of fluoroquinolone-resistant *Campylobacter* (46). This may explain the persistency of fluoroquinolone-resistant *Campylobacter* in the absence of antimicrobial selection pressure in poultry and their environment (47, 48). Changes in the Thr-86-Ile in the gyrA among CIP-R *Campylobacter* recovered from broiler chickens and turkeys in BC needed to be characterized. Vertical transmission and other on-farm sources of contamination (i.e., between farms, other livestock species) and farm-level risk factors such as insufficient downtime/rest period, cleaning, and disinfection have been hypothesized as potential on-farm sources (48) and also warrant investigation.

As described in our previous analysis (31), AMU data were collected from a single grow-out cycle. These data cannot be extrapolated to 1 year of production (e.g., 6 grow-out cycle for broilers and 3–5 cycles for turkeys) due to variations in seasonal antimicrobial use, bird populations, and antimicrobial options (e.g., new products approved for use in poultry). In addition, due to regional differences in disease pressures, agriculture profile variations between provinces (predominant food animal production species), and other operational factors that could potentially drive AMU, these poultry-specific provincial data cannot be extrapolated to national level results. Despite these

limitations, data available at the time of writing of this report permitted exploration of various AMU indicators for detecting trends in AMU and AMR and their utility for future data integration. As the program is progressively strengthened (i.e., by expansion of the farm program in other species such as layers, beef and dairy cattle), robust data could be generated which will subsequently improve the precision of our AMR Ix estimates.

No single AMU or AMR indicator can meet all possible surveillance objectives. Different indicators, in isolation or when integrated with others, highlight different aspects of the complex AMU/AMR issue. Choosing appropriate indicators and then applying them appropriately requires careful consideration of both the data available and the desired objectives. Our findings highlight the utility of AMR Ix in monitoring changes in AMR of organisms of interest to the animal and public health sectors. For integration to be meaningful, data collection, sampling, laboratory techniques, and data management across all sectors must be harmonized. As in any other surveillance program (18), multi-species data may not be available consistently from year to year due to limited resources, which would impact temporal and inter-sectoral analyses. The integration of AMR Ix and an AMU indicator (e.g., mg/PCU) aids in monitoring the effect of AMU reduction interventions, such as the elimination of preventive use of certain antimicrobials (e.g., TIO and ENR which were historically used in an extra-label manner in poultry in Canada), reduction in the use of hatchery-administered antimicrobials (e.g., GEN and LINC-SPEC), or increasing participation in ABF/RWA and organic production (i.e., no use or use of non-medically-important antimicrobials). This study highlights the importance of an ongoing farm-level data AMU and AMR surveillance for monitoring the impact of industry and government interventions to reduce AMR and to inform enhancements to other existing on-farm food safety, flock health management, and AMU practices (e.g., extra-label drug use reduction).

AUTHOR CONTRIBUTIONS

AA, SG, DFL, and CC conceived the study. RI and RR-S supervised all surveillance components. AA, SG, DFL, and AD validated, analyzed, and interpreted the data. AB, DL, DFL, and CC conceptualized and developed the DDDvetCA standards. AA prepared the initial draft, figures, tables, and appendices. All authors contributed to the writing and editing of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2019.00131/full#supplementary-material

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Quantifying Antimicrobial Use in Dutch Companion Animals

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Antimicrobial resistance (AMR) is an increasing threat, both in human and in veterinary medicine. To reduce the selection and spread of AMR, antimicrobial use (AMU) should be optimized, also in companion animals. To be able to optimize AMU, a feasible method to quantify AMU and information on current AMU are needed. Therefore, a method to quantify AMU was developed, using the number of Defined Daily Doses Animal (DDDA). This method was used to explore applied antimicrobial classes and to identify differences in prescribing patterns in time and between veterinary clinics. Antimicrobial procurement data of the years 2012–2014 were collected retrospectively from 100 Dutch veterinary clinics providing care for companion animals. The mean number of DDDAs per clinic per year decreased significantly from 2012 to 2014. A shift in used classes of antimicrobials (AMs) was seen as well, with a significant decrease in use of third choice AMs (i.e., fluoroquinolones and third generation cephalosporins). Large differences in total AMU were seen between clinics ranging from 64-fold in 2012 to 20-fold in 2014. Despite the relative low and decreasing AMU in Dutch companion animal clinics during the study, the substantial differences in antimicrobial prescribing practices between clinics suggest that there is still room for quantitative and qualitative optimization of AMU.

Keywords: antimicrobial, antibiotic, companion animals, veterinary medicine, defined daily dose, DDDA, prescribing

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INTRODUCTION

Antimicrobial resistance (AMR) is an increasing threat, both in human and in veterinary medicine. Many antimicrobials (AMs) used in veterinary medicine are used in human medicine as well. Due to the close contact between people and their companion animals, the importance of companion animals as potential reservoirs of (multi)-resistant pathogens for humans has received increasing attention (1–5). Besides the potential public health threat, AMR also has a direct impact on animal health and welfare, because of treatment failure. To prevent selection and spread of resistant bacteria and to keep AMs valuable for the future, antimicrobial use (AMU) should be optimized.

From 2008 onwards, AMU in Dutch food producing animals received increasing attention, actions were taken at different levels and AMU was reduced considerably (6–9). Most actions addressed food producing animals, but classification of AMs in 1st, 2nd, and 3rd choice AMs [Werkgroep Veterinair Antibiotica Beleid (Working Party for Policy on Veterinary

Antimicrobials)][1] and legislation on mandatory susceptibility testing for the use of 3rd choice AMs (10) also hold for companion animals.

Risk management of AMR needs to be based on valid and most updated information. Therefore, it is crucial to monitor the amount and types of AMs used in animals. Amounts and types of AMs used in animals have been investigated in several countries, particularly in food producing animals (11–16). Only a few studies describe AMU and prescribing patterns in companion animals (17–21). The majority of studies regarding AMU in companion animals uses total sales or prescription data expressed in kilograms of AMs (18), the mass of active AM substances (by AM class or subclass) in relation to a specified population to express AMU or the number of prescriptions (15, 20–22). These different measurement units make it hard to compare data between these studies. The European Medicines Agency, European Surveillance of Veterinary Antimicrobial Consumption group (EMA ESVAC) has introduced the veterinary Defined Daily Doses for Animals (DDD_{VET}) to objectify the numerator (23, 24). DDD_{VET} is defined as a "technical unit of measurement similar to the Defined Daily Dose (DDD), usually based on recommendations from the Summary of Product Characteristics (SPC) and in some cases based on scientific literature, intended for the purpose of drug consumption studies. DDD_{VET} is assigned per kilogram animal per species per day" (23, 24). According to ESVAC, objective AMU data collection should also be organized for companion animals, rabbit production and aquaculture (25).

The aim of present study was to quantify systemic AMU in Dutch companion animal clinics (2012–2014) using Defined Daily Doses Animal (DDDA) established according to the Dutch authorization of the veterinary medicinal products, to explore applied antimicrobial classes and to identify differences in prescribing patterns in time and between veterinary clinics.

MATERIALS AND METHODS

Study Design and Data Collection

A retrospective survey was performed. The Royal Dutch Veterinary Association (KNMvD) provided contact details of all 1,149 veterinary clinics in the Netherlands which treated companion animals. All these clinics were invited by mail to participate, followed by a reminder after 2 weeks by e-mail. Requested data were clinic population data and antimicrobial veterinary medicinal product (AVMP) procurement data for the subsequent years 2012, 2013, and 2014. Mixed-animal clinics with combined, unspecified procurement data for companion animals and non-companion animals were excluded from the study, because products with a multi-species (companion and food producing animal) registration could not be allocated to specific animal species.

Calculation of DDDAs

In the Netherlands, AMs for veterinary use are on prescription only and sold to companion animal owners (or farmers) by veterinarians exclusively. Therefore, antimicrobial procurement data are supposed to reflect the total amount of AMs used in animals. These procurement data were used to calculate the number of DDDAs per clinic per year ($DDDA_{CLINIC}$). For each year and clinic, the number of ordered packages of AVMPs for systemic use was provided, identified by their unique European Article Number (EAN)-code. To calculate the number of DDDAs per clinic, two variables are needed (13). First, the total animal mass in kilogram that can be treated for 1 day with the amount of AMs prescribed; for every individual AVMP this can be derived from the "DG-standaard" by EAN-code. The DG-standaard is an online Dutch database containing all packages of AVMPs once authorized in the Netherlands, managed by the Netherlands Veterinary Medicines Institute. For every single AVMP package, per species the total animal mass in kilogram that can be treated is defined, preferably based on authorized doses, for cascade use based on comparable AVMPs or literature [SDa (the Netherlands Veterinary Medicines Institute)][2]. This database was initially developed and applied for the monitoring of antimicrobial consumption in the major food producing animal sectors, enabling e.g., benchmarking of farms within sectors. Second, the total weight (in kg) of the clinic animal population at risk to be treated with the AVMP. The latter was estimated based on the clinic animal population represented by the number of dogs, cats and rabbits attending the clinic at least once in a specified 3-year period. The total weight was calculated by multiplying the number of dogs, cats and rabbits with previously established average body weights for dogs (19.1 kg) and cats (4.1 kg) (26), for rabbits the average weight was based upon expert opinion (2.5 kg). For every AVMP, the denominator was determined separately depending on the animal species the AVMP was authorized for. By dividing the two variables for all individual AVMPs and consequently adding up the outcomes, the total number of DDDAs is obtained. This sum of all AVMPs is suitable for comparison between clinics and between consecutive years ($DDDA_{CLINIC}$).

This calculation results in the indicator $DDDA_{CLINIC}$/year that represents the theoretical number of days per year an average animal (dog, cat or rabbit) was treated with AVMPs in the clinic concerned. For example, a $DDDA_{CLINIC}$ in 2014 of 2 implies that the average dog, cat and rabbit in care of this veterinary clinic has received 2 days of AM-treatment in 2014.

Classification of AMU

Classification of AMU in present study (**Table 1**) is according to the Dutch policy on veterinary AMU [Werkgroep Veterinair Antibiotica Beleid (Working Party for Policy on Veterinary Antimicrobials)][1].

[1]WVAB-richtlijn classificatie van veterinaire antimicrobiële middelen. Available: https://www.wvab.nl/

[2]DG-standaard (Dutch database for AVMPs). Available: https://cdn.i-pulse.nl/autoriteitdiergeneesmiddelen/userfiles/Publicaties/sda-standard-operating-procedure-dg-standaard-januari-2015.pdf

TABLE 1 | Classification of veterinary antimicrobials (AMs) in 1st, 2nd, and 3rd choice AMs, according to Dutch policy on veterinary AMU.

Classification	Reasoning	Main classes of AMs
1st choice	Empirical therapy; do not select for (to current knowledge), nor are specifically meant for treatment of ESBL-producing micro-organisms	Tetracyclines, nitroimidazoles, narrow-spectrum penicillins, trimethoprim, sulfonamides, and phenicols
2nd choice	All AMs not classified as 1st or 3rd choice AMs; Use of these AMs might select for ESBL-producing bacteria or is specifically indicated in case of an ESBL-infection	Aminopenicillins (with/without beta-lactamase inhibitors), 1st generation cephalosporins, aminoglycosides and colistin
3rd choice	Highest priority critically important AMs for human medicine according to WHO; By Dutch law restricted to use only in individual animals and after culture and susceptibility testing	Fluoroquinolones, 3rd and 4th generation cephalosporins

Statistical Analysis

$DDDA_{CLINIC}$ data were used to determine the proportion of 1st, 2nd, and 3rd choice AMs, to identify trends in AMU during the study period and to identify differences between clinics. Mixed models were used to explore the variation in AMU over time, both within and between clinics. Models for AMU (total, 1st, 2nd, and 3rd choice) were fitted using PROC GLIMMIX (SAS 9.4, SAS Institute, Inc., Cary, NC, USA) assuming a log-normal distribution and allowed for changes in residual variance over time. Within clinic correlations were modeled using an autoregressive [ARH(1)] model and a random intercept. The year of prescription was included as a categorical covariate and statistical significance was tested for using likelihood ratio-testing, comparing model fit to that of a model that not included this covariate (both fitted using maximum likelihood). $P < 0.05$ were considered statistically significant.

RESULTS

Inclusion of Clinics

In total, 155 veterinary clinics responded and were willing to provide specified antimicrobial procurement data (13.5% of the total number of invited clinics). Because of missing data or Practice Management System (PMS) incapability to properly report the animal population data, 44 clinics were excluded. Procurement data from 111 veterinary clinics (period 2012–2014) were included and analyzed. Data from 11/111 veterinary clinics turned out to be inconsistent or unrealistic, i.e., reporting an unexpectedly high or low number of dogs or cats (about 10-times higher or lower than the average clinic) or AVMPs for food producing animals appeared to be incorrectly ascribed to companion animals. Therefore, results are based on data of 100 participating clinics.

Antimicrobial Use: Changes Over Time and Differences Between Clinics

The mean number of DDDAs per clinic per year ($DDDA_{CLINIC}$) decreased from 2.33 (±1.46) in 2012 to 1.88 (±1.20) in 2014 (**Figure 1**). Use of 2nd choice AMs also decreased during the study period [0.97 (±0.77) in 2012 to 0.81 (± 0.63) in 2014] as was the case for 3rd choice AMs [0.55 (±0.38) in 2012 to 0.14 (±0.15) in 2014] (**Figure 2**). First choice AMU increased from 0.81 (±0.93) in 2012 to 0.93 (±0.71) in 2014. Mixed model analyses of AMU (log-transformed data) indicated that all differences between 2012 and 2014 were statistically significant.

In 2012 and 2013, 2nd choice AMs were the most frequently used compounds (42 and 46% of total AMU), whereas in 2014, 1st choice AMs were most frequently used (50% of total AMU). With regard to the groups of AMs used, aminopenicillins (with or without clavulanic acid) defined as 2nd choice AMs, represented the largest group in all three consecutive years (2012; 31%, 2013; 36% and 2014; 36% of total AMU). In 2012, the second largest group of AMs consisted of 3rd generation cephalosporins (i.e., cefovecin) (14% of total AMU), in 2013 and 2014 the second largest group consisted of trimethoprim/sulfonamides (11 and 13% of total AMU, respectively) which are 1st choice AMs. The use of fluoroquinolones and 3rd generation cephalosporins (both 3rd choice AMs) decreased from a mean $DDDA_{CLINIC}$/year number of 0.22 and 0.33 (2012) to 0.08 and 0.07 (2014), respectively.

The majority of systemically used AMs were orally administered (2012 66%; 2013 73%; 2014 77%, respectively). However, major part of 3rd choice AMs were applied parenterally (2012 67%; 2013 63%; 2014 55%, respectively), although this distribution is shifting toward more oral use as well.

The $DDDA_{CLINIC}$ numbers varied from year to year and per clinic (**Figures 3**, **4**). From 2012 to 2014, overall $DDDA_{CLINIC}$ numbers from individual clinics ranged from 0.11 (minimum $DDDA_{CLINIC}$, 2013) to 7.5 (maximum $DDDA_{CLINIC}$, 2014). In 2012, the between clinic difference in total AMU was almost 64-fold (**Figure 3**). In 2014, the between clinic difference was smallest and amounted a 20-fold difference between the minimum and maximum $DDDA_{CLINIC}$ (0.37–7.50) (**Figure 4**). An interesting detail in this observation is that a higher minimum $DDDA_{CLINIC}$ caused the drop in the between clinic difference, not a lower maximum $DDDA_{CLINIC}$. Spearman correlations between repeated measures of total AMU for different pairs of years ranged between 0.7 and 0.8. Regarding the use of 3rd choice AMs, the between clinic difference was larger. Five clinics reported no 3rd choice AMU in 2014. The lowest use that was reported accounted for a $DDDA_{CLINIC}$ of 0.001 while the maximum use was 0.70 in the same year, accounting for a 500-fold difference in 3rd choice AMU between clinics in 2014.

Statistical modeling established the observed differences in AMU between clinics by the mixed model analyses of AMU (log-transformed data) with a heterogeneous AR(1) model, a random "clinic" effect and year of prescription as a covariate. For total AMU the residual variances decreased by 26% from 2012 to 2014. However, for 3rd choice AMU the residual variances

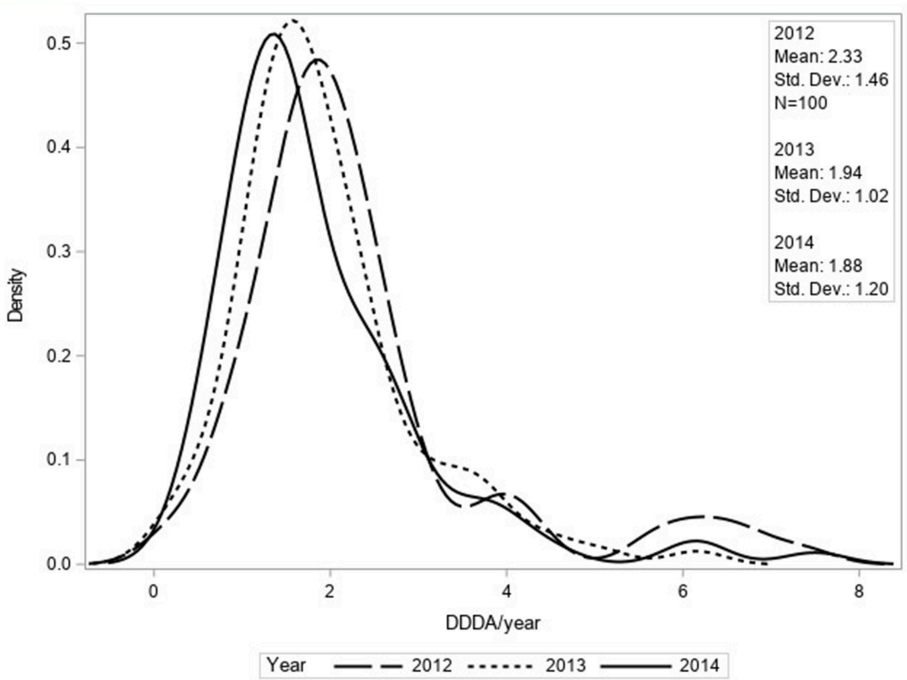

FIGURE 1 | Density function of $DDDA_{CLINIC}$/year for total AMU based upon procurement data of 100 clinics for 2012, 2013, and 2014.

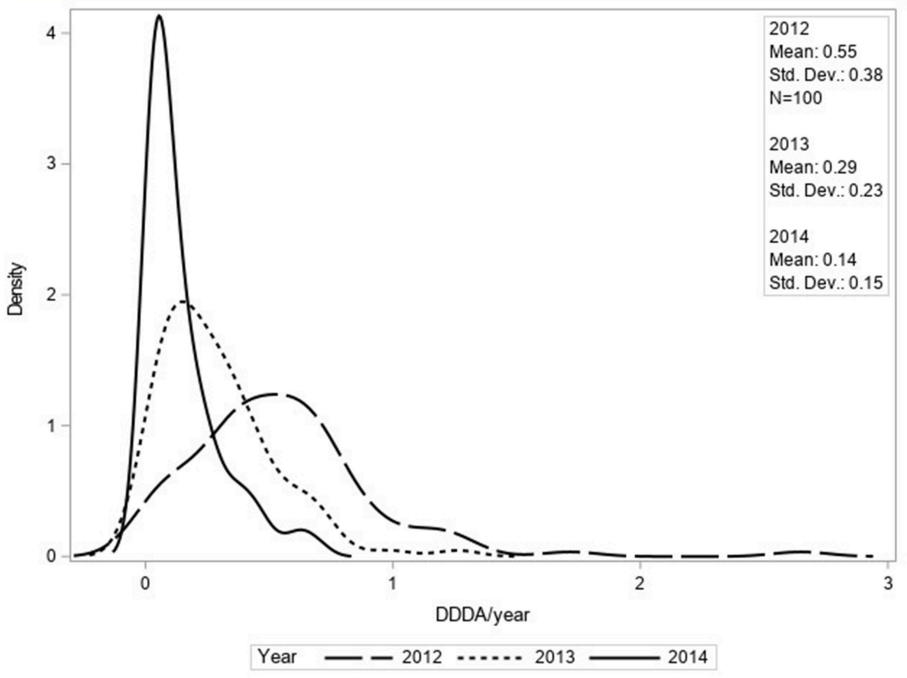

FIGURE 2 | Density function of $DDDA_{CLINIC}$/year for third choice AMU based upon procurement data of 100 clinics for 2012, 2013, and 2014.

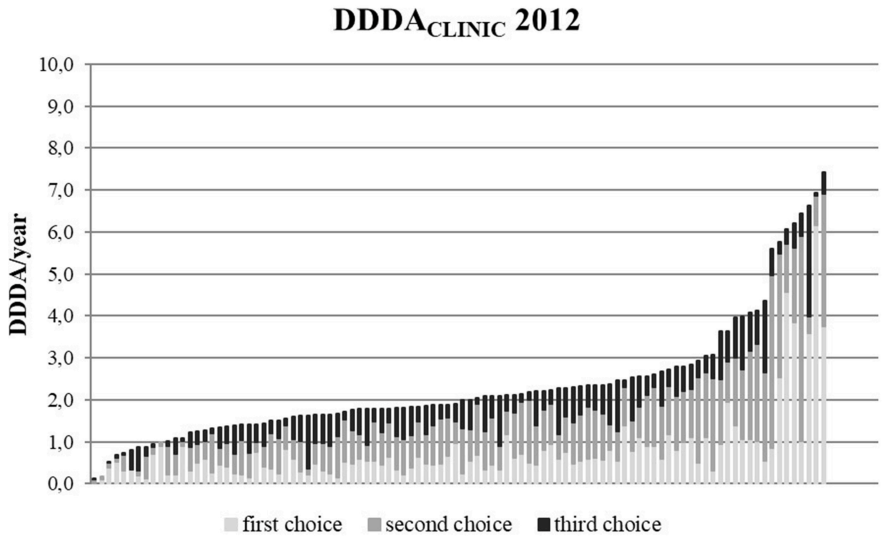

FIGURE 3 | DDDA$_{CLINIC}$ figures for all 100 clinics in 2012, specified for first, second, and third choice antimicrobials, showing the differences in AMU between clinics (based upon procurement data of these 100 clinics).

increased by 102%, indicating that differences between clinics for the use of these AMs became more prominent over time. The estimated correlation between residuals of repeated measures of total AMU for a single clinic for different pairs of years ranged from 0.62 to 0.77 (using log-transformed data), indicating that clear systematic differences exist between practices in AMU.

DISCUSSION

The present study used the number of DDDAs per clinic to express AMU in companion animals. By applying DDDAs dosing differences between AMs due to e.g., the relative potency and differences in pharmacokinetics, are taken into account, as well as dosing differences between species. This measure enables objectified comparison over time and between clinics, even internationally. This measure is adopted in monitoring AMU in food producing animals and endorsed by EMA ESVAC (23). Despite the advantages of a more harmonized way of presenting AMU, there are some disadvantages as well. Disadvantages of using DDDAs are linked to the way DDDAs are calculated. Two variables are needed for this calculation (13) (1) a numerator expressing the total treated animal weight and (2) a denominator expressing the total weight of the clinic animal population. Both variables might be biased. For the numerator this might be the case when an AVMP is authorized for use in more than one animal species. The majority of AVMPs in this study is authorized for more than one of the companion animal species concerned and due to lacking prescription information, it could not be specified whether these AVMPs were prescribed to dogs, cats, and/or rabbits. When it is unknown whether the product has been administered to dogs, cats, or rabbits, the resulting DDDA$_{CLINIC}$ cannot be stratified to specific animal species. At the same time, to be able to determine the total treated animal weight in case of an AVMP that is authorized for more than one companion animal species, the numerator was calculated using the average number of kilograms treated of the species the AVMP was registered for. As an example, if an AVMP was authorized for both dogs, cats and rabbits, the average number of treated kilograms of dogs, cats, and rabbits was calculated as the numerator. In food producing animals, prescription data are collected on farm level making it easier to allocate the AVMPs to specified animal species. Only prescription data (identifying the animal the AVMP was prescribed for) can mitigate this problem of AVMPs authorized for more than one companion animal species. For the denominator, bias might be caused by the total weight of the different animal species and the clinic animal population represented by the number of dogs, cats, and rabbits attending the clinic at least once in a specified 3-year period. In our study, the total clinic animal population of all 100 participating clinics consisted of 228,000 dogs, 228,000 cats, and 25,000 rabbits. These 100 clinics represented 8.7% of 1,149 veterinary clinics treating companion animals in the Netherlands. When these numbers are extrapolated and compared to official estimates on the number of dogs, cats and rabbits in the Netherlands (27), the total number of dogs in the Netherlands is overestimated (correction factor 0.57), the number of cats seems to be estimated correctly (correction factor 0.99) and the number of rabbits is underestimated (correction factor 4.13). The discrepancies between the number of dogs and rabbits registered in veterinary clinics vs. official estimates in the Netherlands (based upon a survey among 7,500 Dutch households) might be explained by the fact that rabbit owners consult a veterinarian less often and dog owners might visit more different clinics (e.g., for a second opinion). The relatively high number of dogs compared to rabbits, might also be explained by

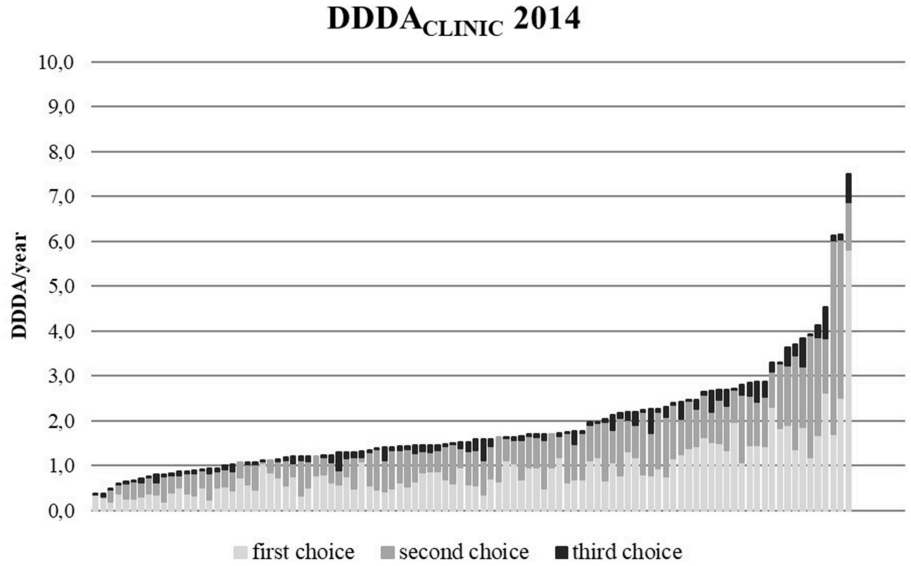

FIGURE 4 | DDDA$_{CLINIC}$ figures for all 100 clinics in 2014, specified for first, second, and third choice antimicrobials, showing the differences in AMU between clinics (based upon procurement data of these 100 clinics).

the fact that rabbit owners mainly visit a veterinarian in case a rabbit is ill, while dog and cat owners might also seek preventive veterinary medicine (e.g., yearly check-ups, vaccinations etc.).

Additional calculations taking above mentioned correction factors into account, result in a mean overall AMU of 2.8 DDDA/year in 2012 (vs. 2.33 without correction factors), 2.34 DDDA/year in 2013 (vs. 1.94 without correction factors), and 2.27 DDDA/year in 2014 (vs. 1.88 without correction factors). Third choice AMU accounted for a mean DDDA/year of 0.69 in 2012 (vs. 0.55), 0.36 in 2013 (vs. 0.29), and 0.19 in 2014 (vs. 0.14), respectively. Although absolute DDDA$_{CLINIC}$ numbers are higher using the correction factors, observed trends and patterns in AMU and differences between clinics remain the same. Regarding the applied denominator per clinic, the absolute DDDA$_{CLINIC}$ values should be interpreted with caution. DDDA is a powerful and objective measure. For comparisons over time and between studies, the denominator should be well-defined.

This study shows a significant decrease of AMU from a mean DDDA$_{CLINIC}$ of 2.33 DDDA/year in 2012 to 1.88 in 2014. This decrease was combined with a clear shift in classes of AMs used. Increased attention for AMU in general and national action plans to establish reduction of AMU in food producing animal sectors, appeared to have affected AMU in Dutch companion animals as well. Not only in the Netherlands, but also in other countries a recent decrease in AM prescriptions in companion animals was reported (20, 21). However, in present study considerable differences in AMU between clinics were seen, suggesting possibilities for optimization of AMU. Given the observation that repeated measures of total AMU from one specific clinic were clearly correlated and substantial between-clinic differences were observed, it would be worth focusing on those clinics with less favorable figures first, although differences between clinics reduced with decreasing use as well. Because 3rd choice AMU was already relatively low, yearly use tended to fluctuate more. Therefore, repeated measures of 3rd choice AMU from one specific clinic appeared less correlated.

Despite a significant reduction in total AMU and especially in 3rd choice AMs [CIAs of highest priority for human medicine according to WHO (28)], the use of these AMs still accounted for 7.7% of total AMU in 2014. However, hard to compare due to using different measurements of AMU, other countries report similar or slightly higher use of highest priority CIAs: in the UK, CIAs of highest priority accounted for just over 6% of AMs used in dogs and 34% in cats (calculated as number of events) (18) and in Australia 8% of the AM courses prescribed belonged to CIAs of highest priority, in which cats were 4.8-times more likely than dogs to receive 3rd generation cephalosporins (21).

Second choice AMs (mainly aminopenicillins and 1st generation cephalosporins) represented the AMs most frequently used in studied Dutch companion animal clinics in 2012 and 2013. Aminopenicillins are categorized as CIAs with a high priority for human medicine (28). These findings are in line with studies in other countries (18, 21).

Total AMU in companion animals is decreasing and relatively low compared to livestock [e.g., in 2014 DDDA$_{NAT}$ for cattle was 2.44, 21.15 for veal calves and 9.52 for pigs, respectively (29)] and AMU in humans [total AMU in the primary care sector of 10.58 DDD/1000 inhabitant days in 2014, corresponding to 3.86 DDD/inhabitant year (30)]. However, regarding the potential selection of ESBL-producing bacteria and regarding the use of 3rd choice AMs, there seems to be room for improvement in the classes and subclasses of AMs used in companion animals. Focus should be on further reduction of 2nd and 3rd choice AMU.

Since January 2013, use of 3rd choice AMs as well as AMs authorized for human use is discouraged by legislation (susceptibility testing is mandatory). Therefore, the amount of

AMs authorized for human use used in veterinary medicine is expected to be low. Based on the present study with veterinary wholesalers' procurement data, the exact amount of AMs authorized for human use (e.g., nitrofurantoin, some clindamycin, and trimethoprim/sulfonamide products) could not be calculated, because data from human pharmacies was not included.

Remarkable differences in AMU between clinics were observed. Overall AMU differed 20-fold in 2014, while for 3rd choice AMs this difference was 500-fold. The residual variance for 3rd choice AMU increased, indicating that differences between clinics with regard to 3rd choice AMU became more prominent. In human primary care the difference in number of antimicrobial courses between Dutch practices was only 5-fold (31). Observed differences in present study might partially be attributed to differences in animal population between clinics. E.g., when clinics treat mainly small or very large breeds, the standardized average animal species weights used for DDDA calculations might not be correct and might cause under- or overestimation of the $DDDA_{CLINIC}$. Also differences in first opinion clinics vs. referral (i.e., secondary or tertiary) clinics, or clinics mainly treating emergency patients might account for observed differences between clinics. However and probably more important, AMU will be determined by prescribing policy and habits within companion animal clinics (e.g., the introduction and implementation of current guidelines regarding AMU) and veterinarian related prescribing habits, e.g., personal preferences in used dosages, frequency of dosing and course lengths as was shown in previous qualitative studies on AMU in companion animal clinics (32–34). The observation of clear and systematic differences between clinics in AMU highlights a potential for further optimization of AMU, eventually leading to smaller differences in AMU between clinics. Therefore, it is of interest to explore underlying factors which may explain differences in AMU between clinics in future studies more in-depth.

Only 8.7% of the 1,149 veterinary clinics treating companion animals were enrolled in present study. The representativeness of these clinics for all Dutch companion animal clinics might be questioned. Participating clinics might have had special interest in AMU and therefore display a more responsible attitude in their AMU compared to non-participating clinics. On the other hand, large differences in AMU between clinics could be observed, indicating that not only clinics with a low AMU were participating. Furthermore, participating clinics were distributed over the whole country. Therefore, the authors believe that the patterns of antimicrobial prescribing are likely to reflect those of the greater population and absolute DDDA numbers can be assumed to provide a reliable lower estimate of AMU across the remainder of the Dutch population of companion animal veterinary clinics.

In conclusion, systemic AMU in Dutch companion animal clinics is decreasing, in particular the use of 3rd choice AMs. However, substantial differences in AMU between clinics could be observed, both in (sub) classes as well as in total amount of AMs used, showing room for improvement.

DATA AVAILABILITY

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

CONTRIBUTION TO THE FIELD

AMR is recognized in human and in veterinary medicine as an increasing threat. The close contact between man and companion animals justifies the recognition of the importance of companion animals as potential reservoirs of (multi)-resistant pathogens for humans. AMR might have a direct impact on animal health and welfare as well. To prevent selection and spread of resistant bacteria and to keep AMs working and effective in the future, AMU should be optimized. In present study, a method was developed, using the number of DDDA, to quantify AMU. With this method used antimicrobial classes were explored and differences in prescribing patterns in time and between veterinary clinics were described. AMU was relatively low and decreasing in participating companion animal clinics, however substantial differences in prescribing practices between clinics suggest that there is still room for quantitative and qualitative improvement. The applied quantification method enables objectified comparison of AMU over time and between clinics, even internationally. Gathered data and developed quantification method will be used in future studies to explore AMU in Dutch companion animals more in-depth, to inform policy makers on AMU developments and to optimize AMU in companion animals.

AUTHOR CONTRIBUTIONS

DH, JW, MvD, and IvG contributed to the concept and design of the study. MvD and IvG collected the data. NH and IvG performed the data analysis. All authors contributed to the writing and revising process of the manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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High-Resolution Monitoring of Antimicrobial Consumption in Vietnamese Small-Scale Chicken Farms Highlights Discrepancies Between Study Metrics

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Chicken is, among farmed species, the target of the highest levels of antimicrobial use (AMU). There are considerable knowledge gaps on how and when antimicrobials are used in commercial small-scale chicken farms. These shortcomings arise from cross-sectional study designs and poor record keeping practiced by many such farmers. Furthermore, there is a large diversity of AMU metrics, and it is not clear how these metrics relate to each other. We performed a longitudinal study on a cohort of small-scale chicken farms ($n = 102$) in the Mekong Delta (Vietnam), an area regarded as a hotspot of AMU, from October 2016 to May 2018. We collected data on all medicine products administered to 203 flocks with the following aims: (1) to describe types and quantities of antimicrobial active ingredients (AAIs) used; (2) to describe critical time points of AMU; and (3) to compare AMU using three quantitative metrics: (a) weight of AAIs related to bird weight at the time of treatment (mg/kg at treatment); (b) weight of AAIs related to weight of birds sold (mg/kg sold); and (c) "treatment incidence" (TI), or the number of daily doses per kilogram of live chicken [Vietnamese animal daily dose (ADDvetVN)] per 1,000 days. Antimicrobials contained in commercial feed, administered by injection ($n = N = 6$), or antimicrobials for human medicine ($n = N = 16$) were excluded. A total of 236 products were identified, containing 42 different AAIs. A total of 76.2% products contained AAIs of "critical importance" according to the World Health Organization (WHO). On average, chickens consumed 791.8 (SEM ±16.7) mg/kg at treatment, 323.4 (SEM ±11.3) mg/kg sold, and the TI was 382.6 (SEM ±5.5) per 1,000 days. AMU was more common early in the production cycle and was highly skewed, with the upper 25% quantile of flocks accounting for 60.7% of total AMU. The observed discrepancies between weight- and dose-based metrics were explained by differences in the strength of AAIs, mortality levels,

and the timing of administration. Results suggest that in small-scale chicken production, AMU reduction efforts should preferentially target the early (brooding) period, which is when birds are most likely to be exposed to antimicrobials, whilst restricting access to antimicrobials of critical importance for human medicine.

Keywords: antimicrobial use, chicken, small-scale farms, metrics, quantification, Vietnam

INTRODUCTION

Antimicrobial resistance (AMR) is a global threat to the health and wealth of nations (1). Antimicrobial usage (AMU) in animal production is regarded as a key driver of AMR in animal populations and a contributor to AMR in humans (2). AMU in animal production has been predicted to increase by 67% from 2010 to 2030 (3), while livestock production may increase by 74% between 1999 and 2030 (4). This increase is mostly driven by increased animal protein consumption in low- and middle-income countries (LMICs).

Chicken meat is the most consumed protein commodity in LMICs because of its comparative advantages. These include the relatively low capital investment and production costs, as well as the lack of religious objections to its consumption (5). In Vietnam, chicken meat currently ranks, after pork, the second most popular type of meat, and by 2020, it is forecast to surpass pork consumption (6).

In 2015, the World Health Organization (WHO) launched its Global Action Plan on AMR, with one of its key objectives being the development and enhancement of monitoring systems for AMU worldwide (7). However, measuring AMU in animal production in LMICs is often challenging due to the large numbers of small-scale farming units, high disease incidence, access of antimicrobials "over the counter," and generally loose regulatory framework (8). According to the Vietnamese official census (2018), of 245M chickens, only 26.1% corresponded to chickens raised in industrial systems (9), with the remainder corresponding to chickens raised in backyard and small-scale (semi-intensive) commercial farms.

AMU can be measured using a large diversity of metrics (10), and the choice of one metric over the other may lead to inconsistent results (11). Several studies have highlighted a very high level of AMU in Vietnamese chicken production, in terms of both frequency and quantities. A study in 210 poultry farms in northern Vietnam reported the use of 45 different antimicrobial active ingredients (AAIs) (12). A cross-sectional study in the Mekong Delta region indicated that, excluding feed, farmers used approximately 470 mg of AAIs to raise one chicken (13). In terms of treatment intensity, AMU in chicken flocks in a neighboring Mekong Delta province (Tien Giang) was 371 defined daily doses (DDD) per 1,000 chicken-days (14). Factors associated with such a high amount of AMU include ease of access to antimicrobials (i.e., density of veterinary drug shops) and the presence of disease and mortality in flocks, which has been described as very high (15).

However, most published studies in Vietnam (and in other LMICs) on AMU to date are based on cross-sectional study designs (i.e., a one-off visit) focused on the prevalent small-scale farm units. Since many farmers do not keep accurate records on AMU, they are likely to be prone to recall biases (16).

Using longitudinal active surveillance on a large cohort of small-scale commercial chicken flocks, we aimed (1) to describe the types of health-supporting products used, with a focus on antimicrobial active ingredients (AAIs); (2) to describe the critical time points for antimicrobial use (AMU) during the production cycle; and (3) to compare AMU using three common metrics of AMU in chicken production in the Mekong Delta of Vietnam. Detailed information about the types and timing of AMU, as well as its magnitude and the relationship between study metrics, is essential in order to improve the design of national/regional monitoring systems. Furthermore, this should help formulate more targeted campaigns aimed at promoting responsible use of antimicrobials among chicken farmers.

MATERIALS AND METHODS

Farms, Flocks, and Data Collection

The study was conducted from October 2016 to May 2018 during the baseline (observational) phase of a research project (17). Chicken farm owners of two districts (Cao Lanh and Thap Muoi) in the province of Dong Thap (Mekong Delta of Vietnam) were randomly selected from the official farm census held by the veterinary authorities (Sub-Department of Animal Health and Production of Dong Thap, SDAH-DT). These two study districts were chosen based on the availability of qualified veterinary staff to conduct the study. The two chosen districts have, on average, a human population of 331 and 354 chickens per square kilometer (2011); these figures are close to the average for the whole Mekong Delta region (410 humans and 478 chickens per square kilometer) (2011).

Farm owners registered in the census ($n = 207$) were convened and introduced to the project. Farmers intending to raise chickens in flocks of >100 chickens were invited to join the study prospectively as soon as they restocked their follow-on cycle. Project staff provided participating farmers with purposefully designed record books organized by week, where they were requested to record in detail the quantities of all health-supporting products used (including antimicrobial-containing products). Farmers were also asked to keep all packages (bottles, sachets, etc.) of any products purchased/used in their flock in a dedicated container. Study farms were visited four times during each flock production cycle to review the product containers (i.e., active ingredients, function, concentration, and instructions for use) and to verify the collected data. All data (commercial product names and quantities used) were entered into a database using a web-based application. The information

collected included number of chickens present in the flock each week and the number of chickens that died over the week. From these data, the flock cycle (cumulative) incidence of mortality was calculated for each production cycle by dividing the total number of birds that died during the period from restocking to sale by the total number of birds restocked for that cycle. A total of 203 flocks that completed at least one entire cycle (from 1-day-old chick until all chicken sold) raised in 102 farms were investigated. Of the 102 farms, 33 (32.3%) completed one cycle, 40 (39.2%) completed two cycles, 19 (18.6%) completed three cycles, 8 (7.8%) completed four cycles, and 2 (19.6%) completed five cycles. Recruited flocks ranged between 100 and 1,530 chickens at restocking. The median flock size at restocking was 300 [Interquartile range (IQR) 200–495]. The median duration of one production cycle was 18 [IQR 16–20] weeks, and the median cumulative mortality over the whole production cycle of flocks was 14.1% [IQR 6.8–29.2].

Description of Health-Supporting Medicinal Products

All health-supporting medicinal products were identified by their composition, and those products containing antimicrobials were singled out. They were described by type (human or veterinary medicine), composition (antimicrobial active ingredient only or mixed with other substances), number of active ingredients, administration route (drinking water, feed, injection), and formulation (powder, liquid). AAIs were classified based on the World Organisation for Animal Health (OIE) list of antimicrobial agents (18).

Timing of Antimicrobial Usage

The probability of a flock being medicated by age (production week) was calculated by dividing the total number of flocks where at least one antimicrobial-containing product was administered by the total number of flocks observed in the same week. In order to investigate potential seasonal variations in AMU, a Lexis diagram was created, with both the probabilities of AMU by production week and week calendar time plotted. A generalized logistic model was fitted with flock identity as the clustering variable and age and calendar week (sine and cos transformed) as covariates. The timing of AMU was investigated for the 20 most commonly used AAIs. The distribution of times of usage of each AAI from week 1 to week 21 (last week of AMU) was plotted.

Quantification of Antimicrobial Usage

The total live weight (body mass in kilograms) of chickens present in each flock at each week was calculated from the number of chickens present in the flock and their estimated weight. The latter was based on weekly weightings of 10 randomly-selected chickens from each of 11 representative flocks, from week 1 until week 22 of their production cycle (**Supplementary Data 1**). The amounts of AAI administered were calculated from farmers' records. The following two weight-based metrics were calculated: (1) weight of active ingredient related to the weight of bird at the time of treatment (mg/kg at treatment) and (2) weight of active antimicrobial active ingredient given over the whole production cycle related to weight of chickens sold (mg/kg sold). This was estimated from the number of chickens present in the flock and their weight at the time of sale. The instructions for mixing the products in water and/or feed (dilution factor) and the estimated daily water and feed consumption were used to estimate for each AAI the daily dose (in mg) associated with treating 1 kg of chicken (ADDvetVN). The weekly water consumption was estimated from the daily intake of a standard meat type pullet at an ambient temperature of 32°C (225 ml per kilogram of live chicken) (19); the weekly feed consumption was estimated from published data related to native Vietnamese layer pullets (i.e., 63.4 g daily per kilogram of live chicken) (20). The expressions used for the calculation of the above metrics are provided in **Supplementary Material S1**.

The number of ADDvetVN of each AAI administered on any given week to each flock (nADDvetVN) was inferred from the amounts of antimicrobial products consumed. The total nADDvetVN administered was divided by the duration of the cycle (in weeks) and multiplied by 1,000. This "treatment incidence" (TI) can be interpreted as the number of days (per 1,000 days) when one chicken is treated.

For antimicrobial products containing two or four AAIs, the number of doses (nADDvetVN) assigned to each AAI contained in the product was calculated as the total number of doses associated with the product divided by two or four, respectively. Products administered through the parenteral route (injection) and human medicines (tablets) were excluded, since the number of chickens receiving injection was not recorded, and guidelines for preparation of human medicines were not available. In addition, antimicrobials contained in purchased commercial feeds were not included in the analyses since they contained ambiguous formulations. Quantitative AMU metrics at the flock level were compared using Pearson's correlation coefficient (PCC). We calculated the mean and coefficient of variation of ADDvetVN values corresponding to AAIs present in Vietnamese antimicrobials and compared them with the DDDvet values defined for poultry by the European Medicines Agency (21).

RESULTS

Health-Supporting Products

A total of 619 different health-supporting products were identified among the 203 flocks investigated, of which 236 (38.1%) contained antimicrobials (**Table 1**). The most common non-antimicrobial health-supporting products ($n = 383$) consisted (in decreasing order) of vitamins/minerals (21.5%), digestive enzymes (8.1%), vaccines (3.7%), coccidiostats (3.6%), electrolytes (3.6%), anthelminthics (2.9%), and interferon/immunoglobulins (0.5%). Of the 112 "other" categories of product, most (∼80%) were anti-inflammatory/anti-pyretic products (i.e., paracetamol, prednisolone).

Of the 236 antimicrobial-containing products, 176 (74.5%) contained only AAIs (apart from excipient), whereas 25.5% contained AAIs mixed with other substances (i.e., vitamins, mineral, electrolytes, anti-inflammatory, and anti-pyretic

TABLE 1 | Summary of health-supporting products used by study flocks.

Type of product	No. of products (n = 619) (%)	Farms (n = 102) (%)	Flocks (n = 203) (%)	Weeks (n = 3,663) (%)
Antimicrobial-containing	236 (38.1)	100 (98.0)	192 (94.5)	933 (25.5)
Non-antimicrobial	383 (61.9)	102 (100)	202 (99.5)	2,128 (63.3)
Vitamins/minerals	133 (21.5)	99 (97.1)	189 (93.6)	1,428 (67.1)
Probiotics	50 (8.1)	86 (84.3)	157 (77.7)	942 (44.3)
Vaccines	23 (3.7)	102 (100)	203 (100)	784 (29.4)
Coccidiostats	22 (3.6)	76 (74.5)	137 (67.8)	304 (14.3)
Electrolytes	22 (3.6)	63 (61.8)	100 (49.5)	299 (14.1)
Anthelminthics	18 (2.9)	49 (48)	71 (35.1)	96 (4.5)
Interferon/immunoglobulins	3 (0.5)	88 (86.3)	144 (71.3)	293 (13.8)
Other (unclassified)	112 (18.1)	81 (79.4)	139 (68.8)	517 (24.3)

TABLE 2 | Description of antimicrobial-containing products administered to 203 chicken flocks.

Category	Sub-category	Products (n = 236) (%)	Farms (n = 102) (%)	Flocks (n = 203) (%)	Week (n = 3,663) (%)
Type of product	Animal medicine	220 (93.2)	100 (98.0)	191 (94.1)	697 (19.0)
	Human medicine	16 (6.8)	6 (5.9)	9 (4.4)	32 (0.9)
Composition	AAI only	176 (74.6)	92 (90.3)	169 (83.2)	629 (16.9)
	AAIs mixed with other substances	60 (25.4)	87 (85.3)	162 (79.8)	448 (12.2)
No. of AAIs per product	One	94 (39.9)	78 (76.5)	135 (66.5)	359 (9.8)
	Two	141 (59.7)	100 (98.0)	190 (93.6)	697 (19.0)
	Four	1 (0.4)	1 (1.0)	1 (0.5)	3 (0.1)
Administration route	Oral	227 (96.2)	100 (98)	192 (95.5)	928 (25.3)
	Oral—water	209 (88.9)	98 (96.1)	191 (94.1)	860 (23.7)
	Oral—feed	21 (8.9)	31 (29.4)	35 (17.2)	190 (5.2)
	Injection	6 (2.5)	13 (12.7)	14 (6.9)	19 (0.5)
Type of formulation	Powder	215 (91.1)	100 (98.0)	191 (94.1)	889 (24.3)
	Liquid	21 (8.9)	36 (35.3)	43 (21.2)	73 (1.9)

AAI, antimicrobial active ingredients.

substances). A total of 141 (59.7%) products contained two AAIs, and 1 (0.4%) contained four AAIs. Overwhelmingly, 227 products (96.2%) were intended for oral administration and 215 products (91.1%) were intended for powder-based formulations (**Table 2**). A total of 16 human medicine products were used by 4.4% of the study flocks. Antimicrobials were used in 25.5% observation weeks (n = 3,663).

Description of Antimicrobial Active Ingredients

A total of 42 different AAIs belonging to 13 classes were identified (**Table 3**). A total of 180 (76.2%) products contained antimicrobials of critical importance according to the WHO (22). Of those, 132 (55.9%) products contained AAIs of critical importance ("highest priority") and 91 (38.5%) products contained critically important ("high priority") antimicrobials. The most common AAI used were colistin (25.8% products, 83.7% flocks), followed by oxytetracycline (15.7%; 76.4%), tylosin (13.6%; 36.9%), doxycycline (11%; 30%), and amoxicillin (10.2%, 24.6%) (**Table 3**). Antimicrobials for human use consisted of tablets containing amoxicillin and tetracycline AAI (three products each); ampicillin, chloramphenicol, ciprofloxacin, and sulfaguanidine (two products each); and cefotaxime (one product). **Supplementary Material S2** includes the list of all AAIs contained in all antimicrobial products investigated.

Antimicrobial Use by Week

A Lexis diagram displaying the probability of AMU of flocks by production age and calendar time (weeks) is shown in **Figure 1**. The probability of AMU decreased with the age of the flock (from 0.76 in week 1, 0.41 in week 2, and 0.02 in week 21). From the Lexis graph, there was an indication of increased AMU during certain calendar periods (peaks in December 2016, June 2017, and February 2018). However, when both variables were fit into the same logistic model with the probability of AMU as an outcome, only the age of the flock (weeks) was significant (data not shown). A median of 5.0 [IQR 2.25–10.0] products and 6.0 [IQR 3.0–10.0] AAIs were used in each flock cycle.

TABLE 3 | AAIs administered to study flocks.

Antimicrobial class	AAI	Products ($n = 236$) (%)	Farms ($n = 102$) (%)	Flocks ($n = 203$) (%)	Weeks ($n = 3,663$) (%)
Aminoglycosides*	Neomycin	17 (7.2)	33 (32.4)	43 (21.2)	85 (3.1)
	Gentamicin	15 (6.4)	41 (40.2)	60 (29.6)	87 (3.2)
	Streptomycin	8 (3.4)	30 (29.4)	41 (20.2)	79 (2.9)
	Spectinomycin	7 (3)	10 (9.8)	12 (5.9)	18 (0.6)
	Apramycin	1 (0.4)	3 (2.9)	3 (1.5)	3 (0.1)
	Any aminoglycoside	50 (21.2)	69 (67.6)	115 (56.7)	259 (9.7)
Amphenicols	Florfenicol	13 (5.5)	24 (23.5)	27 (13.3)	40 (1.5)
	Thiamphenicol	3 (1.3)	20 (19.6)	27 (13.3)	36 (1.3)
	Chloramphenicol	2 (0.8)	2 (2.0)	5 (2.5)	15 (0.5)
	Any amphenicol	18 (7.6)	40 (39.2)	53 (26.1)	90 (3.4)
1st- and 2nd-gen. cephalosporins	Cefadroxil	1 (0.4)	1 (1.0)	1 (0.5)	2 (0.1)
	Cefotaxime	1 (0.4)	1 (1.0)	1 (0.5)	1 (0.0)
	Cefalexin	1 (0.4)	1 (1.0)	1 (0.5)	1 (0.0)
	Any 1st and 2nd gen. cephalosporin	2 (0.8)	2 (2.0)	2 (1.0)	4 (0.2)
Diaminopyrimidines	Trimethoprim	17 (7.2)	31 (30.4)	39 (19.2)	72 (2.7)
Lincosamides	Lincomycin	13 (5.5)	16 (15.7)	21 (10.3)	32 (1.2)
Macrolides**	Tylosin	32 (13.6)	48 (47.1)	75 (36.9)	160 (6.0)
	Tilmicosin	7 (3)	20 (19.6)	24 (11.8)	37 (1.3)
	Erythromycin	6 (2.5)	16 (15.7)	18 (8.9)	27 (1.0)
	Spiramycin	6 (2.5)	11 (10.8)	12 (5.9)	15 (0.5)
	Kitasamycin	1 (0.4)	1 (1.0)	1 (0.5)	1 (0.0)
	Josamycin	1 (0.4)	2 (2.0)	2 (1.0)	4 (0.1)
	Any macrolide	51 (21.6)	57 (55.9)	91 (44.8)	227 (8.5)
Penicillins*	Amoxicillin	24 (10.2)	43 (42.2)	50 (24.6)	87 (3.2)
	Ampicillin	17 (7.2)	27 (26.5)	38 (18.7)	78 (2.9)
	Any penicillin	41 (17.4)	56 (54.9)	91 (44.8)	164 (6.2)
Pleuromutilins	Tiamulin	1 (0.4)	1 (1)	1 (0.5)	1 (0.0)
Polypeptides**	Colistin	61 (25.8)	94 (92.2)	170 (83.7)	413 (15.5)
	Enramycin	1 (0.4)	1 (1.0)	1 (0.5)	1 (0.0)
	Any polypeptide	61 (25.8)	94 (92.2)	170 (83.7)	414 (15.5)
Quinolones/fluoroquinolones**	Enrofloxacin	13 (5.5)	32 (31.4)	45 (22.2)	76 (2.8)
	Flumequine	9 (3.8)	12 (11.8)	16 (7.9)	27 (1.0)
	Norfloxacin	2 (0.8)	7 (6.9)	9 (4.4)	13 (0.4)
	Ciprofloxacin	2 (0.8)	2 (2.0)	3 (1.5)	5 (0.2)
	Marbofloxalin	1 (0.4)	1 (1.0)	1 (0.5)	1 (0.0)
	Any quinolone	27 (11.4)	42 (41.2)	66 (33.5)	122 (4.6)
Sulfonamides	Sulphamethoxazole	7 (3.0)	26 (25.5)	34 (16.7)	68 (2.5)
	Sulfadimidine	6 (2.5)	8 (7.8)	9 (4.4)	11 (0.4)
	Sulfadimethoxine	6 (2.5)	14 (13.7)	16 (7.9)	21 (0.8)
	Sulfaguanidin	2 (0.8)	2 (2.0)	4 (2.0)	11 (0.4)
	Sulfadiazine	2 (0.8)	2 (2.0)	2 (1.0)	4 (0.1)
	Sulfamethoxypyridazine	1 (0.4)	2 (2.0)	2 (1.0)	4 (0.1)
	Sulfachloropyridazine	1 (0.4)	1 (1.0)	1 (0.5)	1 (0.0)
	Sulfamethazine	1 (0.4)	1 (1.0)	1 (0.5)	1 (0.0)
	Sulfathiazole	1 (0.4)	1 (1.0)	1 (0.5)	1 (0.0)
	Any sulfonamide	25 (10.6)	45 (44.1)	60 (29.6)	118 (4.4)
Tetracyclines	Oxytetracycline	37 (15.7)	87 (85.3)	155 (76.4)	332 (12.4)
	Doxycycline	26 (11.0)	42 (41.2)	61 (30.0)	129 (4.8)
	Tetracycline	6 (2.5)	7 (6.9)	10 (4.9)	28 (1.0)
	Any tetracycline	69 (29.2)	93 (91.2)	173 (85.2)	474 (17.8)
Unclassified	Methenamine	1 (0.4)	15 (14.7)	23 (11.3)	31 (1.1)

*Critically important antimicrobial classes according to the World Health Organization (WHO) are highlighted: *High priority, **Highest priority.*

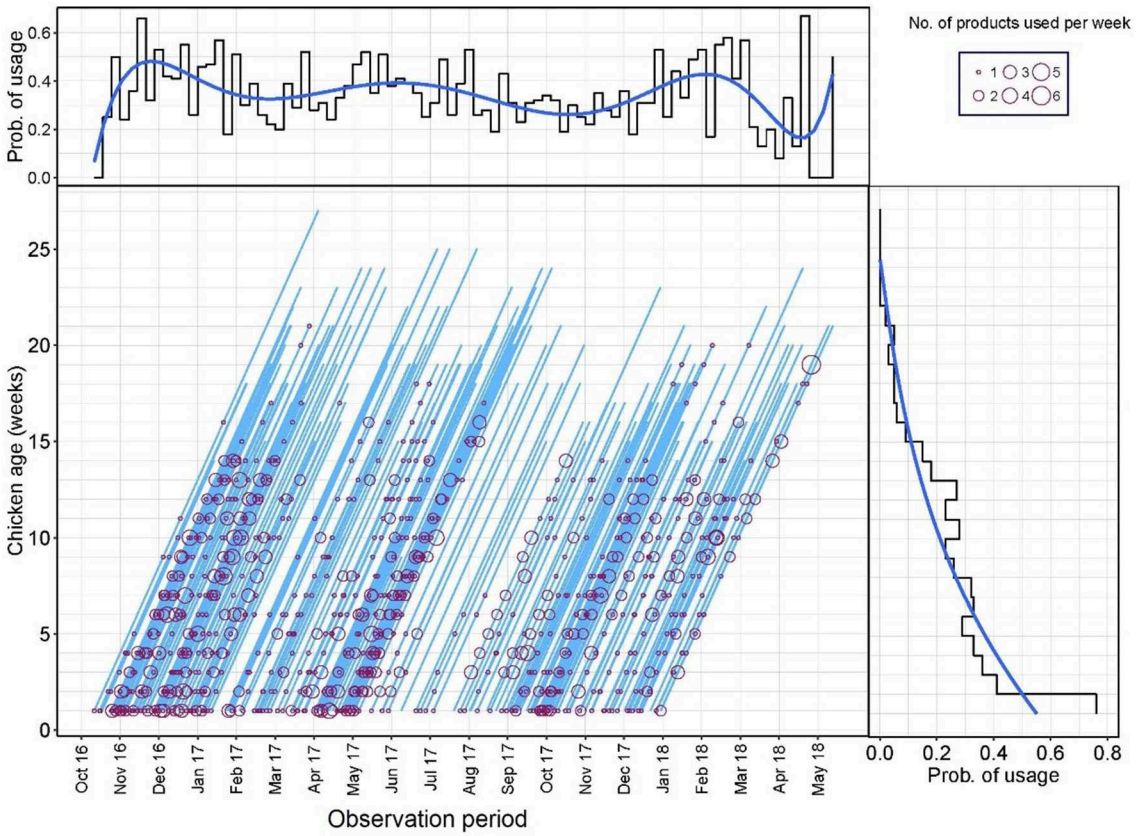

FIGURE 1 | Lexis diagram and probability of antimicrobial use (AMU) (Yes/No) by production week and calendar week during the study period.

Timing of Antimicrobial Use

In terms of timing of use, the AAIs used earlier in the production cycle were oxytetracycline [median timing of use, 2 weeks (IQR 1–5)], thiamphenicol [median 2.0 (IQR 1.0–6.0)], and colistin [median 3 (IQR 1.0–7.0)]. Tilmicosin [median 9 (IQR 6.0–12.0)], flumequine [median 9.0 (IQR 7.0–13.0)], and tetracycline [median 10.0 (IQR 6.0–12.0)] were the three AAIs that were administered latest to study flocks (**Figure 2**).

Quantification of Antimicrobial Use

Chicken flocks were administered a mean of 791.8 (±16.7) mg AAI per kilogram of chicken at treatment time [median 512 mg (IQR 264–1,094)] and 323.4 (±11.3) mg per kilogram of chicken sold [median 134 mg (IQR 62–279)]. The mean TI was 382.6 (±5.5) ADDs per 1,000 days [median 290 (IQR 125–583) per 1,000 days] (**Figure 3**). These calculations excluded AAIs contained in commercial feed, injectables, or human medicine antimicrobials. The data were quite skewed in all three metrics, with the mean being always greater than the median value. In terms of mg/kg at treatment, the upper 25% quantile of flocks accounted for 60.7% of total use. In addition, 23 (12.0%) flocks used more than 1,000 doses per 1,000 chicken days. For the "mg/kg sold" metric calculation, 9/203 (4.4%) flocks were excluded, since they experienced 100% mortality and therefore no live chickens were sold from such flocks.

Tetracyclines were the most used antimicrobial class reflected in both metrics: 285.1 mg/kg at treatment (SEM ±23.4) and a TI of 150.9 (±9.3) per 1,000 days. In terms of mg/kg at treatment, the highest magnitude of AMU corresponded to oxytetracycline 231.5 mg (29.2%), methenamine 105.8 mg (13.2%), and amoxicillin 48.7 mg (6.2%); in contrast, the highest TI corresponded to colistin 145.5, oxytetracycline 141.8, and enrofloxacin 16.1 (**Table 4**).

Correlation Between Antimicrobial Use Metrics

Figure 4 shows the three correlation plots between each pair of the three AMU metrics used. Correlation was highest between "mg/kg sold" and "mg/kg at treatment" (PCC = 0.457; $p < 0.001$) (moderate positive relationship). The metric "mg/kg at treatment" was weakly correlated with "treatment incidence" (PCC = 0.212; $p < 0.001$). There was no correlation between TI and mg/kg sold metric (PCC = 0.008; $p = 0.223$). The proportion of flocks with high mortality (≥14.1%) was significantly greater among flocks with higher than average AMU expressed with the mg/kg sold metric (0.64 vs. 0.34, $\chi^2 = 15.52$; $p < 0.001$). In the case of the other two metrics, there were no significant differences in mortality between high and low AMU users (both $p > 0.407$).

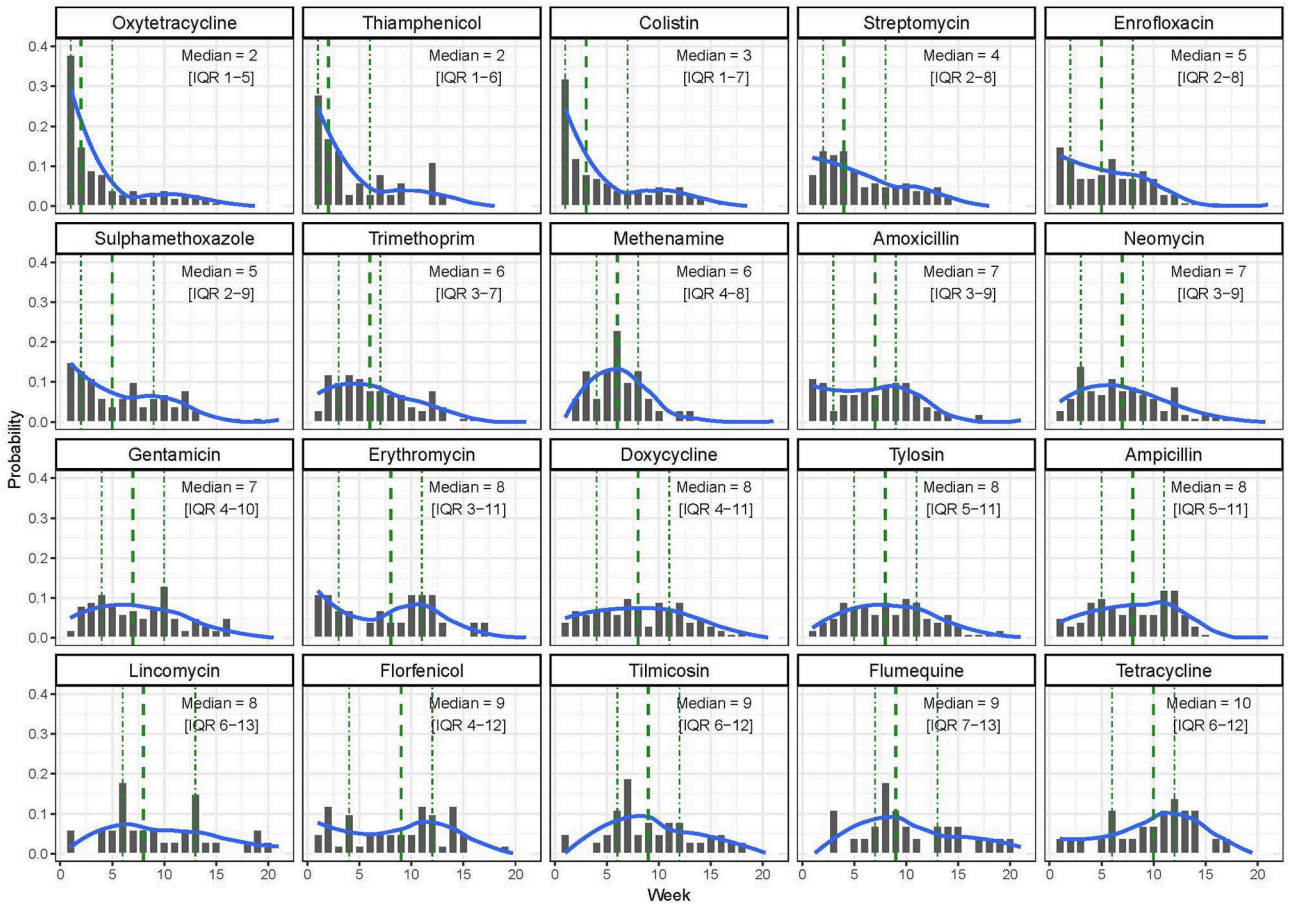

FIGURE 2 | Distribution of the timing of use of the 20 most common antimicrobial active ingredients (AAIs) by week among study flocks.

Vietnamese Animal Daily Dose for Chicken Production

The mean ADDvetVN corresponding to each of 37 AAIs was calculated from 223 different veterinary medicine products (**Supplementary Material S3**). ADDvetVN values ranged from 4.4 mg (sulfamethazine) to 320.6 mg (methenamine). However, most of the values were lower than 50 mg (35/38 AAI). A very high coefficient of variation (>100%) was also observed in several AAIs such as colistin, gentamicin, doxycycline, trimethoprim, tylosin, neomycin, spectinomycin, sulfadimidine, and florfenicol. There were 27 AAIs with data on DDDvet for poultry available in the European Union (EU). Of those, 14/27 antimicrobials from Vietnamese products had lower ADDs, while 13/27 had higher ADDs. Notably, the values of several DDDvet from the EU (i.e., spectinomycin, tylosin, ampicillin, and spiramycin) were four to five times higher than ADDvetVN.

Antimicrobial Use by Antimicrobial Active Ingredients

Figure 5 shows the correlation between TI and weight-based metrics (mg/kg at treatment and total weight of antimicrobials ignoring population treated) by AAI (**Supplementary Material S3**). The two metrics were moderately correlated (PPC > >0.480, $p < 0.001$ in both cases). However, the greater deviation from perfect correlation was observed for those AAIs with very low (i.e., colistin) or very high (i.e., methenamine) ADDvetVN values (5.2 and 320.6 mg/kg chicken, respectively). Comparing antimicrobials with similar TI, such as methenamine and spectinomycin (i.e., both ∼1 ADD per 1,000 chicken-days), given that the former has a much higher ADDvetVN value (320.6 mg/kg) than the latter (33 mg/kg), this results in quantitatively larger estimates for methenamine in terms of "total amounts (grams) of active ingredient" (**Figure 5**, right).

DISCUSSION

Our study deliberately focused on small-scale commercial farming systems. In doing so, we excluded both larger industrial (broiler) and backyard production systems. The small-scale commercial chicken sector represented here, alongside industrial broiler production, is increasingly important in Vietnam: from

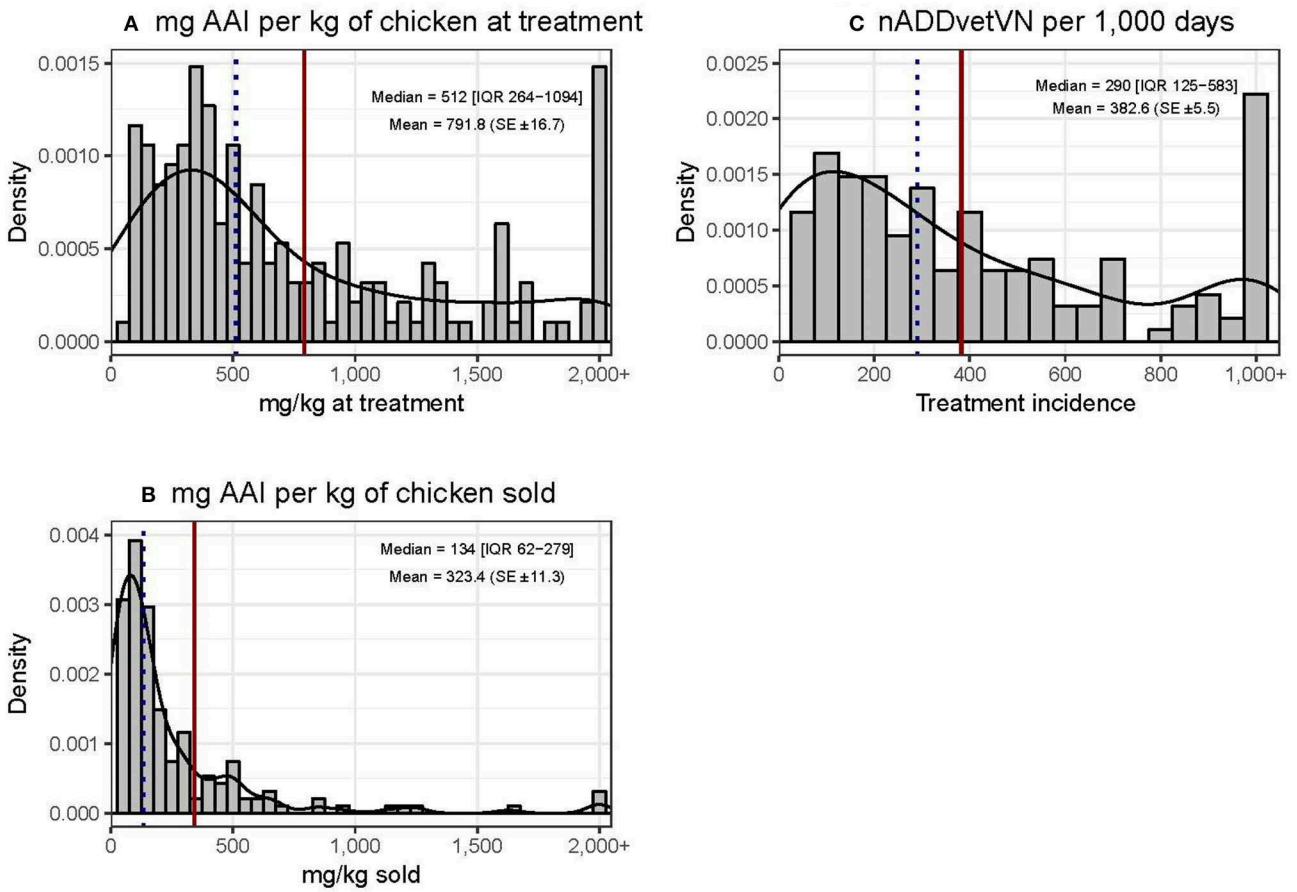

FIGURE 3 | Distribution of quantitative metrics of AMU in study flocks. Dotted black line: median. Solid red line: mean.

2011 to 2016 the number of registered units raising more than 100 chickens has experienced a 41.5% increase (23).

Using three different metrics, this study provided an accurate characterization of AMU in small-scale chicken flocks in the Mekong Delta of Vietnam, an area regarded as a hotspot of AMU. AMU levels were 791.8 (SEM ±16.7) mg of AAI per kilogram at treatment and 323.4 (SEM ±11.3) mg per kilogram sold. In terms of TI, chicken flocks were treated on average 382.6 days (SEM ±5.5) per 1,000 days. These results excluded antimicrobials included in purchased commercial feed formulations and a few antimicrobial products that were administered through the injectable route or human medicine antimicrobials products. In Vietnam, antimicrobials included in commercial feed have been quantified to be in the order of 77.4 mg per kilogram of live chicken raised in a previous study. In terms of TI, chickens in our study consumed three times more than global average levels (estimated in 138.0 doses per 1,000 chicken-days) (10).

It is particularly concerning that around three quarters (76.2%) of the products examined contained AAIs of "critical importance," and over half (55.9%) contained at least one AAI of critical importance (highest priority) according to the WHO (i.e., colistin, quinolones, and macrolides). The magnitude of colistin use is of particular concern, since this is one of the antimicrobials of last resort for hospital-acquired infections in humans (24). Colistin was found either alone or in combination with other antimicrobials such as oxytetracycline, ampicillin, neomycin, tylosin, enrofloxacin, etc. A possible reason for its popularity is its low cost, since it is an older-generation antimicrobial. Most (∼60%) antimicrobial-containing products were formulated with two AAIs. This scenario is different from European countries, where one active ingredient is allowed, except for a few drugs that are always formulated as combination (i.e., trimethoprim and sulphonamides) (21). In a small percentage of flocks (4%), we found that farmers had used chloramphenicol, an antimicrobial that has been banned for almost two decades in the country (25). In 2% of farms, ciprofloxacin (also banned for use in animal production) had also been used. We found a large number of farms that administered more doses than those technically necessary over the life of the flock. We believe that this is a reflection of errors in the preparation resulting in excessive concentration of the AAI during the early phases, since the costs of administering antimicrobials in small birds is relatively lower.

Results from this study highlight significant discrepancies between metrics. Relating AMU to chicken weight at treatment results in estimates of a magnitude two to three times higher than

TABLE 4 | Amounts of AAIs used through the oral route in study flocks.

Antimicrobial class	AAI	Mean AMU by flock (±SEM)		
		mg/kg at treatment	mg/kg sold	Treatment incidence
Aminoglycosides	Neomycin	38.0 (±16.4)	14.7 (±5.9)	4.4 (±1.1)
	Gentamicin	12.5 (±3.2)	6.3 (±3.5)	2.1 (±0.4)
	Streptomycin	22.5 (±10.5)	14.3 (±16)	6.0 (±1.3)
	Spectinomycin	2.2 (±3)	0.6 (±0.7)	1.0 (±1.0)
	Apramycin	0.5 (±1.1)	1.2 (±7.2)	<0.1 (±nc)
	Josamycin	0.9 (±3.2)	7.5 (±68)	<0.1 (±nc)
	Total aminoglycosides	75.7 (±5.9)	37.5 (±24.2)	13.5 (±2.7)
Amphenicols	Florfenicol	7.3 (±3.7)	9.4 (±12.1)	1.9 (±0.8)
	Thiamphenicol	26.2 (±12.5)	4.4 (±3.7)	3.1 (±0.6)
	Chloramphenicol	nc	nc	nc
	Total amphenicols	33.5 (±6.6)	13.8 (±1.2)	5.0 (±1.6)
1st and 2nd gen. cephalosporins	Cefadroxil	0.5 (±nc)	<0.1 (±nc)	<0.1 (±nc)
	Cefotaxime	nc	nc	nc
	Cefalexin	<0.1 (±nc)	<0.1 (±nc)	<0.1 (±nc)
	Total	0.5 (±nc)	<0.1 (±nc)	<0.1 (±nc)
Diaminopyrimidines	Trimethoprim	25.7 (±nc)	11.7 (±nc)	4.3 (±nc)
Lincosamides	Lincomycin	3.2 (±nc)	2.3 (±nc)	1.4 (±nc)
Macrolides	Tylosin	34.8 (±8.5)	27.7 (±17.3)	6.5 (±1.2)
	Tilmicosin	25.9 (±19.2)	20.9 (±25.4)	7.8 (±4.6)
	Erythromycin	12.2 (±16.1)	5.7 (±12.3)	3.8 (±2.9)
	Spiramycin	1.5 (±1.4)	0.2 (±0.5)	1.1 (±0.5)
	Kitasamycin	<0.1 (±nc)	0.4 (±nc)	<0.1 (±nc)
	Josamycin	0.9 (±3.2)	7.5 (±68)	< 0.1 (±nc)
	Total	75.3 (±7.9)	62.0 (±10.4)	19.2 (±7.5)
Penicillins	Amoxicillin	48.7 (±24.7)	25.8 (±28.7)	14.4 (±3.4)
	Ampicillin	11.1 (±6.1)	5.5 (±4)	1.5 (±0.8)
	Total	59.8 (±13.2)	31.3 (±17.5)	15.9 (±7.5)
Pleuromutilins	Tiamulin	<0.1 (±nc)	<0.1 (±nc)	<0.1 (±nc)
Polypeptides	Colistin	41.6 (±5.7)	8.8 (±1.6)	145.8 (±4.6)
	Enramycin	<0.1 (±nc)	<0.1 (±nc)	<0.1 (±nc)
	Total	41.6 (±3.5)	8.8 (±0.9)	145.8 (±5.9)
Quinolones/Fluoroquinolones	Enrofloxacin	24.1 (±8.4)	7.4 (±4.6)	16.1 (±2.6)
	Flumequine	5.4 (±3.2)	3.4 (±2)	0.6 (±0.2)
	Norfloxacin	6.4 (±6.5)	2.4 (±3.5)	1.1 (±0.8)
	Ciprofloxacin	nc	nc	nc
	Marbofloxalin	nc	nc	nc
	Total	35.9 (±5.6)	13.2 (±4.8)	17.8 (±7.8)
Sulfonamides	Sulphamethoxazole	30.2 (±1.2)	11.7 (±15.1)	3.6 (±0.6)
	Sulfadimidine	4.1 (±4.8)	2.3 (±2.5)	0.1 (±nc)
	Sulfadimethoxine	13.5 (±27.7)	2.4 (±2)	1.9 (±1.4)
	Sulfaguanidin	nc	nc	nc
	Sulfadiazine	2.4 (±10)	0.7 (±4.8)	0.2 (±0.3)
	Sulfamethoxypyridazine	0.5 (±2)	0.3 (±0.8)	<0.1 (±nc)
	Sulfachloropyridazine	<0.1 (±nc)	<0.1 (±nc)	<0.1 (±nc)
	Sulfamethazine	0.7 (±nc)	<0.1 (±nc)	1.0 (±nc)
	Sulfathiazole	<0.1 (±nc)	<0.1 (±nc)	<0.1 (±nc)
	Total	51.4 (±9.5)	17.4 (±5.1)	4.9 ±1.4)

(Continued)

TABLE 4 | Continued

Antimicrobial class	AAI	Mean AMU by flock (±SEM)		
		mg/kg at treatment	mg/kg sold	Treatment incidence
Tetracyclines	Oxytetracycline	231.5 (±21.0)	43.7 (±9.8)	141.8 (±4.6)
	Doxycycline	42.6 (±13.3)	14.0 (±3.4)	7.5 (±1.2)
	Tetracycline	7.4 (±46.8)	7.9 (±52.5)	1.6 (±4.0)
	Total	285.1 (±23.4)	65.6 (±27.9)	150.9 (±9.3)
Unclassified	Methenamine	105.8 (±nc)	58.0 (±nc)	1.1 (±nc)
Total		791.8 (±16.7)	323.4 (±11.3)	382.6 (±5.5)

nc, not calculated.

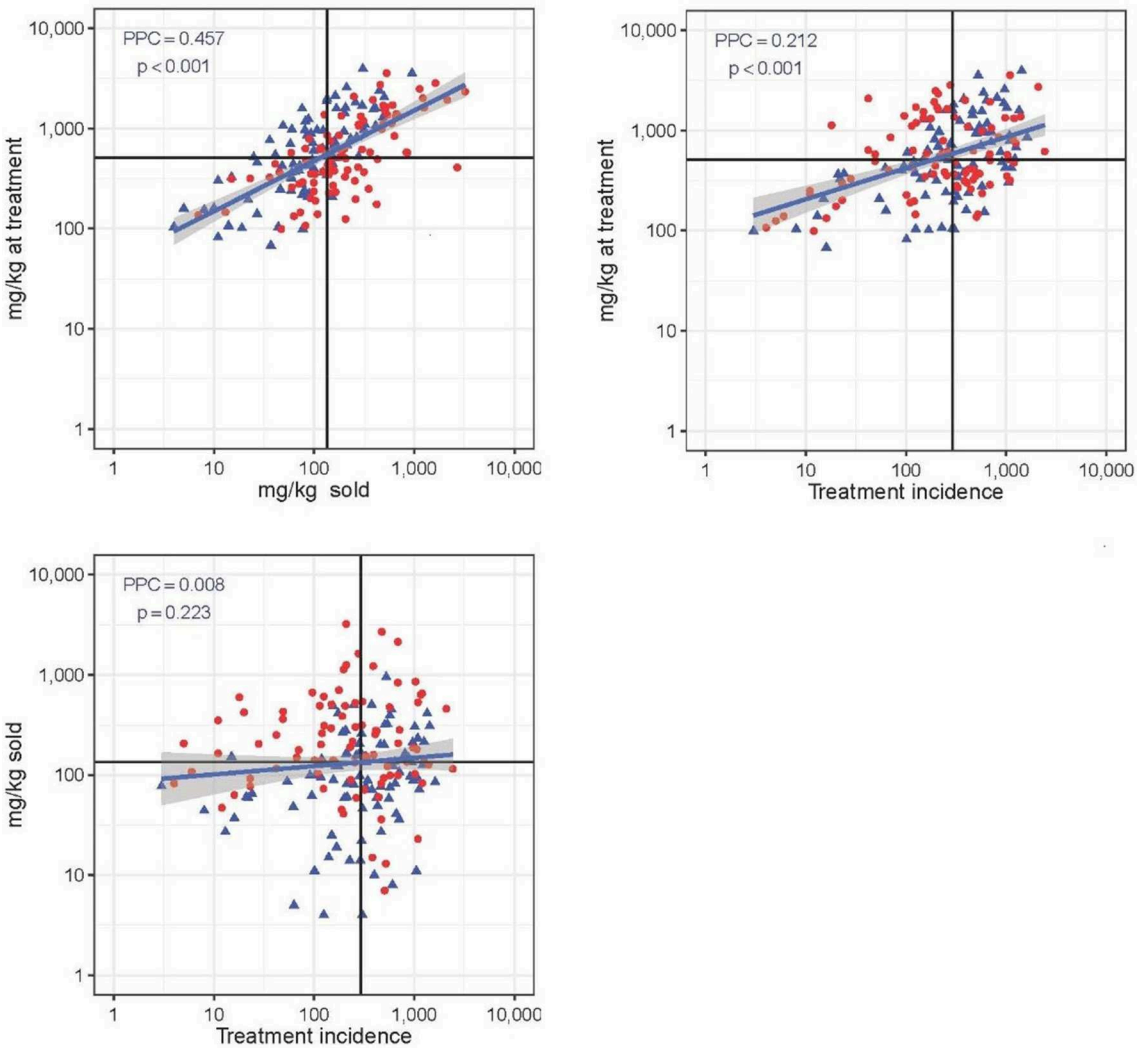

FIGURE 4 | Correlation between three quantitative AMU metrics ("mg/kg at treatment," "mg/kg sold," and "treatment incidence"). Solid black lines represent the median value of each metric. PCC is Pearsons's correlation coefficient. Red dot: flock with high (≥14.1%) mortality; blue dot: flock with low (<14.4%) mortality. Note the log 10-transformed scale for easier visualization.

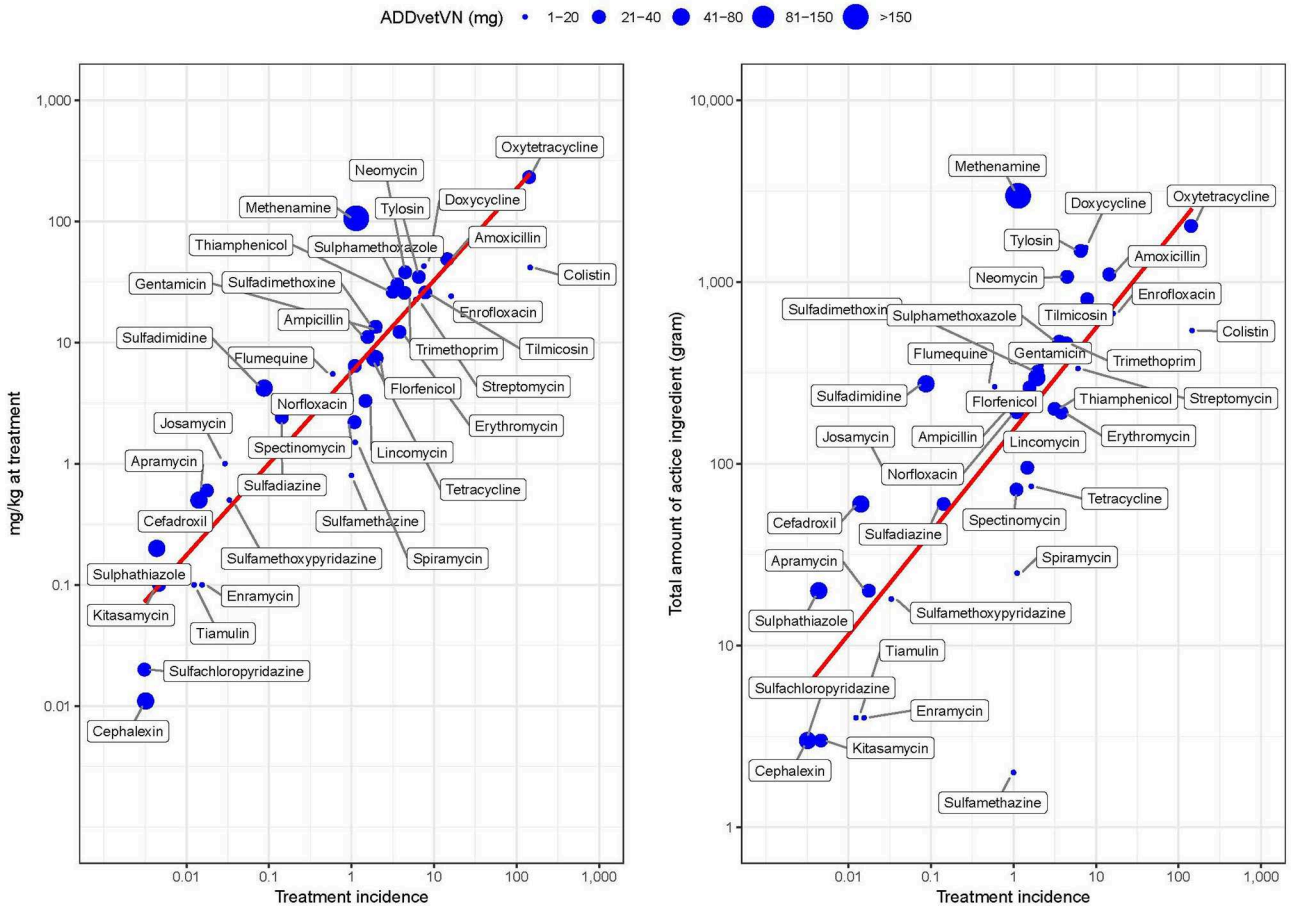

FIGURE 5 | Relationship between study metrics by AAI in study flocks. The size of the dot is proportional to the magnitude of the Vietnamese animal daily dose (ADDvetVN) for each AAI (in mg). Note the log 10-transformed scale for easier visualization.

relating AMU to chicken weight at the end of the production cycle. The "mg/kg at treatment" metric was largely influenced by the timing of AMU, with higher values resulting from administration of the product early in the production cycle (i.e., brooding), therefore resulting in larger estimates. The "mg/kg at treatment" use is expected to always be higher than "mg/kg sold," since the weight at the end of production is typically the highest. This latter metric was, however, largely affected by mortality, with flocks experiencing high mortality having considerably higher AMU estimates due to the smaller denominator in such flocks. If national estimates of AMU were to be calculated from production data, it is therefore essential to factor in the high levels of mortality that are typical of each production system. The "treatment incidence" metric is the most balanced overall metric, since it incorporates the variability associated with the variable strength of the AAIs administered. However, a challenge associated with the latter is the definition of a "daily dose," given that most antimicrobial products included guidelines for both prophylactic and therapeutic use, and information on the actual preparation procedures used by the farmer (dilution factor) was not collected. Indications for prophylactic use involve mixing the product with approximately half the strength of indication for therapeutic use. In addition, most products contain two AAIs, and each AAI amounted to half a theoretical daily dose in the overall calculation. The major discrepancies observed between weight-based and dose-based metrics can be explained because of differences in strength of different AAIs, timing of use, and variable mortality. In situations where AAIs characterized by large technical units are used, calculations using weight-based metrics will result in the overestimation of results using weight-based metrics over treatment incidence metrics.

We report differences in the timing of usage of different antimicrobials. Some antimicrobials, such as tetracycline and tilmicosin, have withdrawal times of over 1 week (26), and in several cases were administered late in the production cycle. This probably explains the high rate of detection of macrolide and tetracycline residues (10.3% each) in chicken meat samples purchased from the study area (27).

The study highlighted a huge diversity of AAIs used by small-scale chicken farmers. In Vietnam, about 10,000 products are currently licensed for veterinary use (28, 29), and ∼50% contain AAIs (author's observation). We established

the Vietnamese "animal daily dose" for antimicrobials used in chicken production (ADDvetVN). Athough our calculations of ADDvetVN were based on the indication displayed in the label for therapeutic purpose, most values were still lower than the DDDvet from the European Union, and for several AAIs (i.e., spiramycin, ampicillin) they were four to five times lower. In addition, many products included a recommendationa for prophylactic use, where the product is diluted by a factor of two, and the AAI is therefore administered at an even lower concentration. This is a concern, since such low doses may contribute to increased generation of AMR (30).

We are confident that farmers did provide an honest record of all antimicrobial products used and that the data collected in our study accurately represent AMU in these small-scale farming systems. This was possible since project staff were not perceived to judge farmers' practices negatively. However, obtaining longitudinal high-resolution data required several visits during the production cycle, and a considerable degree of both farmer and research staff commitment. Therefore, these types of studies may not be feasible at a large (i.e., national surveillance) scale, unless considerable resources are dedicated. We understand that the small-scale sector is the target of the largest quantities of AMU in Vietnam, and most of this use is for prophylactic purposes (15). This category of farmers should be the focus of policy makers to reduce excessive AMU in animal production. In Vietnam, most antimicrobials used in animal production are procured by farmers in licensed veterinary pharmacies. Because of this, we believe that setting up monitoring systems at these retail points, coupled with detailed animal production statistics (to be collected at local level), would represent a much more cost-effective surveillance system for AMU compared with conducting farm surveys.

Results highlight the need for training chicken farmers to improve their awareness on AMR while discouraging prophylactic use of antimicrobials, particularly during the brooding period. Such training should emphasize the need to improve day-old chick quality and farming practices (biosecurity, cleaning and disinfection, brooding management, and vaccination). Furthermore, in view of the high usage levels of AMU of critical importance (high priority), we recommend authorities to introduce phased restrictions, starting with those AAIs belonging to the highest priority group.

DATA AVAILABILITY

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

The ViParc project has been granted ethics approval by the Oxford Tropical Research Ethics Committee (OXTREC) (Minimal Risk) (Ref. 5121/16).

AUTHOR CONTRIBUTIONS

JC-M, PP, and NC conceived the study. NC, BK, BH, HT, and NV developed data collection methods and carried out field visits. NC, NV, MC, BT, and DP contributed to data analyses. NC, GT, MC, and JC-M contributed to manuscript writing-up and editing.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2019.00174/full#supplementary-material

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Pharmaceutical Prescription in Canine Acute Diarrhoea: A Longitudinal Electronic Health Record Analysis of First Opinion Veterinary Practices

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Canine acute diarrhoea is frequently observed in first opinion practice, though little is known about commonly used diagnostic or therapeutic management plans, including use of antimicrobials. This retrospective observational study utilised electronic health records augmented with practitioner-completed questionnaires from 3,189 cases (3,159 dogs) collected from 179 volunteer veterinary practices between April 2014 and January 2017. We used multivariable analysis to explore factors potentially associated with pharmaceutical agent prescription, and resolution of clinical signs by 10 days post-initial presentation. Use of bacteriological and/or parasitological diagnostic tests were uncommon (3.2% of cases, 95% confidence interval, CI, 2.4–4.0), though systemic antimicrobials were the most commonly prescribed pharmaceutical agents (49.7% of cases, 95% CI 46.1–53.2). Such prescription was associated with haemorrhagic diarrhoea (odds ratio, OR, 4.1; 95% CI 3.4–5.0), body temperature in excess of 39.0°C, or moderate/severe cases (OR 1.3, 95% CI 1.1–1.7). Gastrointestinal agents (e.g., antacids) were prescribed to 37.7% of cases (95% CI 35.4–39.9), and were most frequently prescribed to vomiting dogs regardless of presence (OR 46.4, 95% CI 19.4–110.8) or absence of blood (OR 17.1, 95% CI 13.4–21.9). Endoparasiticides/endectocides were prescribed to 7.8% of cases (95% CI 6.8–9.0), such prescription being less frequent for moderate/severe cases (OR 0.5, 95% CI 0.4–0.7), though more frequent when weight loss was recorded (OR 3.4, 95% CI 1.3–9.0). Gastrointestinal nutraceuticals (e.g., probiotics) were dispensed to 60.8% of cases (95% CI 57.1–64.6), these cases less frequently presenting with moderate/severe clinical signs (OR 0.6, 95% CI 0.5–0.8). Nearly a quarter of cases were judged lost to follow-up (n=754). Insured (OR 0.7, 95% CI 0.5–0.9); neutered (OR 0.4, 95% CI 0.3–0.5), or vaccinated dogs (OR 0.3, 95% CI 0.3–0.4) were less commonly lost to follow-up. Of remaining dogs, clinical signs were deemed resolved in 95.4% of cases (95% CI 94.6–96.2). Provision of dietary modification advice and gastrointestinal nutraceuticals alone were positively associated with resolution (OR 2.8, 95% CI 1.3–6.1); no such

associations were found for pharmaceutical agents, including antimicrobials. Hence, this study supports the view that antimicrobials are largely unnecessary for acute diarrhoea cases; this being of particular importance when considering the global threat posed by antimicrobial resistance.

Keywords: health informatics, antimicrobial resistance, companion animal, electronic health record, pharmacosurveillance, acute canine diarrhoea, haemorrhagic diarrhoea

INTRODUCTION

Acute diarrhoea commonly affects dogs (1). Whilst the majority of cases are generally mild and self-limiting, some can be life threatening (2–7). Aetiology is complex, including a range of non-infectious lifestyle factors, such as a history of scavenging or being fed home-cooked diets (1, 5, 6). Zoonotic (e.g., *Campylobacter, Salmonella, Giardia* spp.) and non-zoonotic (e.g., canine parvovirus, canine enteric coronavirus) pathogens have also been implicated (6, 8–14), though the precise role some of these play remains of debate (8, 15–18). A range of therapeutic options are available, either targeting potential infectious agents and/or clinical signs (2, 5). Together this creates a complex clinical decision-making environment for practitioners when first presented with such cases, further compounded by relatively infrequent use of diagnostic testing (5).

Antimicrobial prescription, as a management strategy, is a particular focus for research due to the increasing threat posed by antimicrobial resistance (19). Antimicrobial prescription has been recorded in between 45 and 70% of canine diarrhoea cases (2, 5, 7, 20, 21), with prescription being most frequent in cases presenting with pyrexia or haemorrhagic diarrhoea (2, 5). These findings most likely reflect a perception that such clinical signs increase likelihood of infectious process involvement and/or intestinal mucosal compromise, increasing risk of bacteraemia (15). However, recent case-control studies of canine acute haemorrhagic diarrhoea syndrome (AHDS) have questioned whether antimicrobial therapy has an impact on odds of recovery in non-septic patients (10, 22), and indeed whether antimicrobials should be prescribed at all (10, 16, 23).

In addition to antimicrobial prescription, management strategies frequently encompass other pharmaceutical agents both as primary diarrhoea therapies (24), or to manage associated clinical signs (2). The potential utility of gastrointestinal nutraceuticals (including prebiotics, probiotics, adsorbents, and motility modifiers) has also attracted recent attention (25, 26), though evidence of *in vivo* efficacy remains limited (25).

The complex and often undetermined aetiology of acute canine diarrhoea, as well as the range of therapeutic or management interventions available, provides a natural opportunity to more fully understand factors that might drive complex clinical decision-making in practice, as well as which of these decisions might impact outcome. This study aimed to combine electronic health record (EHR) and questionnaire data collected from a large network of UK veterinary practices to explore factors associated with the decision to prescribe pharmaceutical agents or dispense nutraceuticals to dogs presenting with acute diarrhoea.

MATERIALS AND METHODS

Data Collection

This longitudinal retrospective study analysed electronic health records (EHRs) collected from 179 volunteer veterinary practices (347 sites) situated in the United Kingdom (UK) that participate in the Small Animal Veterinary Surveillance Network (SAVSNET) and utilise Robovet practice management software (Vet Solutions Ltd.). A veterinary practice was defined as a single business, whereas "sites" included all branches that comprised an individual veterinary practice. SAVSNET hold ethical approval from the University of Liverpool (RETH000964); data collection protocols are more fully described elsewhere (4). Briefly, EHRs were collected from consultations where a booked appointment was made to see a veterinary professional (veterinary surgeon or veterinary nurse) between 1 April 2014 and 31 January 2017. Every consultation was classified by the consulting veterinary professional into one of ten categories indicating the main reason that the animal presented and the main presenting complaint (MPC) (21). In addition to the MPC, a further questionnaire was completed in a random selection of consultations by the attending veterinary professional (**Table 1**). Consultations which had been classified into the "gastroenteric" MPC, which also had an associated completed questionnaire attached were selected for inclusion in this study.

A case was defined as a dog presenting for investigation of acute diarrhoea (**Table 1**, question 1) of 2 days or less duration (**Table 1**, question 5), where the relevant consultation was the first time the animal had presented for investigation of that diarrhoeic episode (**Table 1**, question 4). Consultations were selected for presence of diarrhoea but not at the exclusion of other clinical signs. In addition to a range of signalment data (e.g., age, sex, breed etc.), the MPC, and the associated questionnaire responses, each EHR also included a text-based product description and free text clinical narrative. The latter was manually interrogated to summarise animal body temperature (if recorded). Each EHR also contained a vaccination history; animals were defined as currently vaccinated if they had received a vaccination of any composition within 3.5 years preceding the relevant consultation date (27).

Pharmaceutical, Nutraceutical, or Veterinary Professional Advice Identification

Pharmaceutical agent prescriptions were identified and classified via the semi-structured text-based product description field of the EHR (28). Antimicrobials and anti-inflammatories were further classified by authorised administration route as

TABLE 1 | Questions provided to consulting veterinary professionals in ∼10% of consultations (selected at random) where they had selected "gastrointestinal" as the main reason the owner presented the animal to the practice.

Question	Answer options
1. Please indicate the clinical signs present	Diarrhoea without blood
	Diarrhoea with blood
	Vomiting without blood
	Vomiting with blood
	Melaena
	Weight loss/failure to gain weight
	Poor appetite
	Other
2. If diarrhoea was present how would you describe it?	Small intestinal diarrhoea
	Large intestinal diarrhoea/colitis
	Mixed pattern
	No diarrhoea
	Don't know
3. Please indicate disease severity	Mild illness i.e., normal apart from GI disease
	Moderately ill
	Severely ill/debilitated
4. How does this consultation relate to this episode of illness?	First presentation
	Revisit/check-up
	Don't know
5. How long approximately has the pet had this episode of illness?	Up to 2 days
	Between 3 days and 2 weeks
	More than 2 weeks—less than 1 month
	1 month and over
	Don't know
6. What diagnostic options will be used today for this episode of illness?	None
	Faecal parasitology/bacteriology
	Faecal virology
	Virus serology
	Diagnostic imaging
	Haematology/biochemistry
	Serum B12/Folate and/or serum TLI
	Canine/feline specific pancreatic lipase
	Urinalysis
	Other
7. What advice did you give today?	Change of diet
	Fasting
	Admit patient for treatment
	Refer patient
	Check-up in near future
	Other

systemic (oral or injectable forms, hence "systemic") or topical administration (aural, ocular, skin). Five pharmaceutical families commonly prescribed for management of canine gastroenteric disease (5) were selected for further analyses: systemic antimicrobials (excluding topical antimicrobials), systemic anti-inflammatories (excluding topical anti-inflammatories), gastrointestinal agents e.g., antacids, gastro-protectants, anti-emetics etc., endoparasiticides and endectocides, and products used for euthanasia (henceforth, "euthanasia"). Additionally, the product description field was interrogated to identify dispensed gastrointestinal nutraceutical products. These were defined as products not listed as either authorised veterinary or human medicinal products which contained a range of probiotics, prebiotics, kaolin etc., and were marketed for the purpose of aiding diarrhoea resolution.

Case Follow-Up

As the majority of canine self-limiting diarrhoea cases resolve within a week (29), cases were considered as resolved if they did not return to the veterinary practice for a mainly gastroenteric reason (as judged by MPC) between 11 and 30 days post-initial presentation. The clinical narratives for all cases re-presenting for examination between 1 and 10 days post-initial presentation were additionally read to record explicit mention of diarrhoeic clinical sign resolution, and any further pharmaceutical prescriptions provided in this time period. Cases were defined as lost to follow-up if they did not re-present to the veterinary practice at all by 31 January 2018, or were seen again within 10 days post-initial presentation but did not re-present by 31 January 2018.

Statistical Analyses

All analyses were carried out using R language (version 3.4.4). Descriptive proportions and associated 95% confidence intervals (95% CI) were calculated to adjust for clustering (bootstrap method, $n = 5{,}000$ samples) within site,[1] encompassing a range of binary or categorical demographic, clinical sign, pharmaceutical agent prescription, and clinical outcome variables. Median and range were calculated for continuous variables. Following descriptive analyses, univariable and multivariable mixed effects logistic regression were used to model a range of outcomes, using the R package "lme4"[2] Primary outcomes, modelled as binary variables, included resolution of diarrhoea clinical signs (as defined above), and loss to follow-up. The decision to prescribe systemic antimicrobials, systemic anti-inflammatories, gastrointestinal agents, endoparasiticides and endectocides, and to euthanise the dog at initial presentation were also explored, using prescription of such agents as binary independent variables in separate models. A likelihood ratio test (LRT) of a null model indicated that observations were clustered within practice and site; hence both were included as random effects in all models. Univariable regression was first performed, with explanatory variables being retained if a LRT indicated $P \leq 0.20$ against a null model.

In total, 21 binary or categorical explanatory variables were considered. For all models, these included factors related to animal signalment (insurance status, vaccination status, sex, neutered status, microchip status); questionnaire responses (presence of haemorrhagic diarrhoea, melaena, vomiting, decreased appetite, weight loss, diarrhoeic pattern, clinical severity) and categorised body temperature as recorded within the clinical narrative attached to each consultation (interpreted normal or below 39.0°C, 39.0–39.4°C, 39.5–39.9°C, in excess

[1] AOD Package. Available from: https://cran.r-project.org/package=aod
[2] LME4 Package. Available from: https://cran.r-project.org/package=lme4

of 40.0°C, and temperature not recorded), as recorded within the clinical narrative attached to each consultation. Considering clinical severity, due to a low number of severe cases such cases were combined into a single category with moderate cases for all models. Animal's age at consultation was fitted as a continuous explanatory variable; where relevant, polynomial terms were fitted if an LRT indicated significantly improved fit.

For models considering resolution or loss to follow-up only, questionnaire responses indicating that the consulting veterinary professional had provided advice to either modify the animal's diet or fast the animal were also considered. Prescription of pharmaceutical agents or dispensed gastrointestinal nutraceuticals were also included in resolution and loss to follow-up models as binary dependent variables.

In order to take into account the therapeutic complexities surrounding case management, we took three separate approaches to modelling the association of each of the five therapeutic options (e.g., the four investigated pharmaceutical families and gastrointestinal nutraceuticals) and dietary modification advice against case resolution. Firstly, we considered each option/advice regardless of presence of other options e.g., a single case must be prescribed a systemic antimicrobial, but could also be prescribed/dispensed/advised any other option. Secondly, we considered each option/advice in isolation e.g., a single case could only be prescribed a systemic antimicrobial, with no further prescription/dispensing/advice provided. Finally, we also considered dietary advice in combination with each option in isolation e.g., a single case must be provided with dietary modification advice and prescribed a systemic antimicrobial, with no further options provided.

Multivariable analyses underwent step-wise backward elimination to reduce Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC). Two-way interaction terms were assessed for improved multivariable model fit via a combination of AIC and BIC. Multicollinearity was assessed in the final model via use of the Variance Inflation Factor (VIF), available through the R package "car"[3] Odds ratios, confidence intervals, correlation of fixed effects and projected probabilities were calculated utilising the R package "sjPlot"[4] Statistical significance was defined throughout as $P < 0.05$.

RESULTS

Study Population

In total, 12,455 questionnaires were completed for canine patients (11,589 unique dogs) with a gastroenteric MPC, of which 3,192 questionnaires (3,162 unique dogs) fitted the acute diarrhoea case definition (two days or less duration and first presentation for examination). Three dogs were removed where a spurious date of birth was recorded (e.g., 1st January 1900). Hence, 3,189 diarrhoea cases (involving 3,159 unique dogs) collected from 179 veterinary practices (347 sites) were included in analyses (**Figure 1**). Of these retained cases, 50.2% (95% CI, 48.3–52.1) were recorded as male; 62.2% of male cases were neutered (95% CI 59.6–64.9), 72.4% of female cases were neutered (95% CI 69.7–75.1), 54.6% of total retained cases were microchipped (95% CI 52.1–57.2), 24.4% of total retained cases were insured (95% CI 22.1–26.7), and 73.6% of total retained cases has been vaccinated within the preceding 3.5 years (95% CI 71.5–75.8). Median age at initial presentation was 4.2 years (range 0.0–18.5).

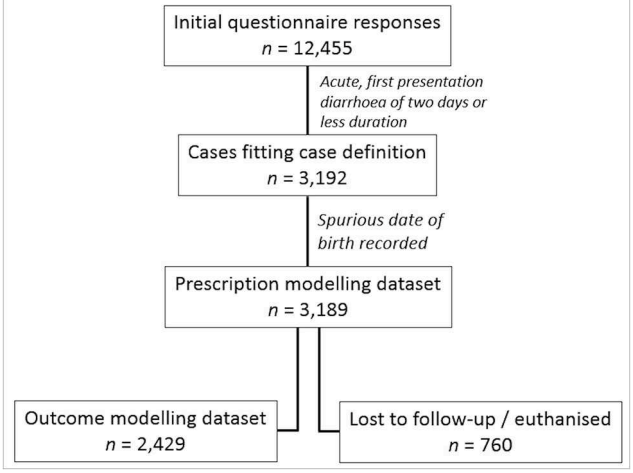

FIGURE 1 | Flow diagram showing case selection procedure for prescription modelling, loss to follow-up, and outcome modelling.

Descriptive Analyses

Cases were considered by clinical severity (**Table 1**, question 3), clinical sign combinations (**Table 1**, question 1), and body temperature as recorded in the clinical narrative. The majority of dogs [n = 1,893; 59.4% of cases (95% CI 57.8–61.0)] initially presented with non-haemorrhagic diarrhoea (**Table 2**). Most cases were recorded as mild (n = 2,665; 83.6% of cases, 95% CI 82.2–85.0), with moderate cases more commonly reporting diarrhoea with blood, vomiting, weight loss, poor appetite, and a mixed diarrhoeic pattern compared to mild cases. Utilisation of diagnostic tests was uncommon (<10% of all cases), with bacteriology and parasitology being the most commonly performed test (3.2% of cases, 95% CI 2.4–4.0). Dietary modification was the most commonly provided advice to dog owners. In total, 1,812 cases explicitly recorded body temperature within the clinical narrative, reporting a median body temperature of 38.6°C (range 36.2–41.3); a further 53 and 3 cases recorded a "normal" or "increased" temperature, respectively without stating a value. Considered together, an interpreted normal or below 39.0°C body temperature was recorded in 58.4% of cases (n = 1,865, 95% CI 56.5–60.4); 39.0°C−39.4°C in 13.1% (n = 418, 95% CI 11.8–14.4), 39.5–39.9°C in 3.5% (n = 110, 95% CI 2.8–4.1), and >40.0°C in 0.9% of cases (n = 30, 95% CI 0.6–1.3). Temperature was not recorded or interpreted in 23.9% of cases (n = 763, 95% CI 21.9–25.9). A greater proportion of cases were classified as moderate or severe as reported temperature increased (data not presented).

[3]CAR Package. Available from: https://cran.r-project.org/package=car.
[4]sjPlot Package. Available from: https://cran.r-project.org/package=sjPlot

TABLE 2 | Descriptive summary of questionnaire responses for both the entire study population and when stratified by the consulting veterinary professional's assessed case severity, according to questionnaire responses.

Question	Response	All cases (n = 3,189 cases)	Mild case (n = 2,665 cases)	Moderate case (n = 507 cases)	Severe case (n = 17 cases)
		% (95% CI)[a]	% (95% CI)	% (95% CI)	% (95% CI)
1. Clinical signs	Diarrhoea without blood	59.4 (57.8–61.0)	60.2 (58.4–62.0)	55.9 (52.0–59.7)	41.8 (17.4–66.1)
	Diarrhoea with blood	40.6 (39.0–42.3)	39.9 (38.1–41.6)	44.2 (40.4–48.0)	58.5 (34.3–82.7)
	Vomit without blood	33.7 (31.8–35.6)	28.9 (27.0–30.8)	58.8 (54.0–63.6)	41.4 (16.7–66.0)
	Vomit with blood	2.3 (1.7–2.9)	2.0 (1.4–2.5)	3.8 (2.1–5.4)	11.8 (0.0–27.3)
	Melaena	0.4 (0.2–0.6)	0.2 (0.0–0.3)	1.6 (0.5–2.7)	5.9 (0.0–17.0)
	Weight loss[b]	1.2 (0.8–1.6)	0.8 (0.5–1.1)	3.2 (1.4–4.9)	6.0 (0.0–17.2)
	Poor appetite	13.8 (12.4–15.2)	10.3 (8.9–11.6)	31.5 (27.3–35.8)	35.4 (11.9–58.8)
	Other	1.6 (1.0–2.2)	1.2 (0.7–1.7)	3.4 (1.5–5.2)	17.9 (0.0–36.4)
2. Pattern	Small intestinal	32.7 (30.5–34.8)	31.8 (29.5–34.1)	37.5 (32.7–42.4)	29.6 (7.5–51.8)
	Large intestinal	39.0 (36.9–41.1)	41.3 (39.2–43.5)	27.0 (22.6–31.4)	35.4 (12.0–58.8)
	Mixed pattern	19.7 (18.0–21.4)	18.2 (16.6–19.9)	26.8 (22.3–31.2)	22.8 (0.0–46.1)
	Don't know	8.7 (7.4–9.9)	8.6 (7.3–10.0)	8.7 (6.3–11.1)	11.9 (0.0–27.1)
3. Diagnostic options	Total	9.0 (7.7–10.3)	7.7 (6.4–9.0)	13.7 (10.2–17.1)	70.3 (47.9–92.6)
	Bacteriology/parasitology	3.2 (2.4–4.0)	3.2 (2.4–4.1)	2.8 (1.3–4.3)	6.0 (0.0–17.3)
	Faecal virology	0.1 (0.0–0.3)	0.0 (0.0–0.1)	0.4 (0.0–0.9)	5.9 (0.0–17.1)
	Virus serology	0.1 (0.0–0.1)	0.0 (0.0–0.1)	0.2 (0.0–0.6)	0.0 (0.0–0.0)
	Diagnostic imaging	0.9 (0.5–1.3)	0.6 (0.3–1.0)	2.0 (0.5–3.5)	11.7 (0.0–25.8)
	Haematology/biochemistry	2.4 (1.8–3.1)	1.4 (0.9–1.8)	6.1 (3.8–8.4)	52.7 (27.9–77.5)
	Serum B12 and/or TLI	0.1 (0.0–0.2)	0.1 (0.0–0.2)	0.2 (0.0–0.6)	0.0 (0.0–0.0)
	Specific pancreatic lipase	0.6 (0.3–0.9)	0.4 (0.2–0.6)	1.6 (0.4–2.8)	11.8 (0.0–27.4)
	Urinalysis	0.3 (0.1–0.4)	0.3 (0.1–0.5)	0.2 (0.0–0.6)	0.0 (0.0–0.0)
	Other	3.1 (2.4–3.9)	2.9 (2.1–3.6)	4.1 (2.3–5.9)	17.6 (0.0–35.8)
4. Advice	Change of diet	69.8 (67.3–72.4)	71.1 (68.5–73.8)	65.0 (60.2–69.8)	5.6 (0.0–15.5)
	Fasting	19.1 (16.4–21.8)	18.4 (15.5–21.3)	23.0 (18.4–27.5)	5.9 (0.0–17.2)
	Admission	2.3 (1.7–3.0)	1.2 (0.7–1.6)	6.5 (4.2–8.8)	58.7 (34.7–82.7)
	Refer	0.2 (0.0–0.3)	0.1 (0.0–0.2)	0.2 (0.0–0.6)	5.6 (0.0–15.8)
	Check-up	22.9 (20.9–24.9)	20.1 (18.0–22.2)	37.5 (32.9–42.0)	17.0 (0.0–38.8)
	Other	49.4 (46.6–52.2)	50.3 (47.2–53.3)	45.3 (40.9–49.8)	35.8 (12.5–59.1)

[a] *Percentage of cases (95% confidence interval).*
[b] *Weight loss or failure to gain weight.*

Pharmaceutical prescription occurred in 78.4% (95% CI 76.3–80.5) of initial presentations, rising to 81.3% of cases (95% CI 79.5–83.2) within 10 days post-initial presentation (**Table 3**). Systemic antimicrobials were the most commonly prescribed pharmaceutical agent (49.7% of cases at initial presentation, rising to 52.5% within 10 days of initial presentation). Gastrointestinal nutraceuticals were also frequently dispensed (60.8% of cases at initial presentation, rising to 61.7% within 10 days of initial presentation). In total, 4.3% of cases (95% CI 3.4–5.2) had no record of a pharmaceutical agent being prescribed or a gastrointestinal nutraceutical dispensed. Metronidazole represented the most commonly prescribed systemic antimicrobial (47.0% of antimicrobial prescribing cases, 95% CI 41.0–53.1); glucocorticoids the most commonly prescribed systemic anti-inflammatory (81.3% of anti-inflammatory prescribing cases, 95% CI 73.6–89.1); maropitant the most commonly prescribed gastrointestinal agent (44.6% of gastrointestinal prescribing cases, 95% CI 39.9–49.3), and a combination of milbemycins and quinolines were the most commonly prescribed endoparasiticides/endectocides (48.0% of endoparasiticide/ endectocide prescribing cases, 95% CI 41.5–54.5) (see **Supplementary Table 1**).

Pharmaceutical prescription frequency varied by case severity, with systemic antimicrobials largely being prescribed to mild and moderate cases (**Table 3**), and showed considerable variation between cases, particularly in relation to co-prescription (**Table 4**). Systemic antimicrobial prescription was more frequent in cases reporting diarrhoea with blood compared to diarrhoea without blood, regardless of presence or absence of vomiting (see **Supplementary Table 2**).

Factors Associated With Pharmaceutical or Nutraceutical Intervention

No variables were significant on univariable analyses for systemic anti-inflammatory prescription (see **Supplementary Table 3**); hence no further statistical analysis was performed for this prescription category.

TABLE 3 | Descriptive summary of pharmaceutical prescriptions and dispensing of nutraceutical products both at initial presentation and when the subsequent 9 days (inclusive) post-presentation were considered.

Category	All cases	Mild case	Moderate case	Severe case
	% (95% CI)[a]	% (95% CI)	% (95% CI)	% (95% CI)
THERAPY—INITIAL PRESENTATION				
Pharmaceutical agent	78.4 (76.3–80.5)	76.4 (74.0–78.9)	89.4 (86.8–92.0)	58.9 (36.7–81.1)
Systemic antimicrobial	49.7 (46.1–53.2)	48.2 (44.5–51.9)	58.5 (53.4–63.7)	5.9 (0.0–17.2)
Systemic anti-inflammatory	14.2 (10.6–17.8)	14.2 (10.5–17.9)	15.0 (10.1–19.9)	0.0 (0.0–0.0)
Gastrointestinal agent	37.7 (35.4–39.9)	33.3 (31.0–35.6)	60.9 (56.5–65.4)	17.5 (0.3–34.6)
Endoparasiticide and/or endectocide	7.8 (6.8–9.0)	8.7 (7.5–10.0)	3.6 (2.0–5.2)	0.0 (0.0–0.0)
Gastrointestinal nutraceutical	60.8 (57.1–64.6)	63.0 (59.1–66.9)	51.1 (46.0–56.3)	11.9 (0.0–27.5)
Euthanasia/death	0.2 (0.0–0.3)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	35.3 (11.9–58.8)
THERAPY—INITIAL PRESENTATION AND/OR WITHIN 10 DAYS OF INITIAL PRESENTATION				
Pharmaceutical agent	81.3 (79.5–83.2)	79.6 (77.4–81.8)	91.1 (88.7–93.6)	59.0 (37.0–81.1)
Systemic antimicrobial	52.5 (49.1–55.8)	51.2 (47.7–54.6)	61.1 (56.2–66.1)	5.9 (0.0–17.3)
Systemic anti-inflammatory	15.3 (11.6–19.0)	15.4 (11.8–19.1)	15.3 (10.5–20.2)	0.0 (0.0–0.0)
Gastrointestinal agent	39.0 (36.7–41.2)	34.7 (32.3–37.2)	62.0 (57.6–66.4)	17.6 (0.4–34.8)
Endoparasiticide and/or endectocide	9.5 (8.3–10.7)	10.6 (9.2–11.9)	4.3 (2.4–6.1)	0.0 (0.0–0.0)
Gastrointestinal nutraceutical	61.7 (58.1–65.4)	63.7 (59.8–67.5)	53.4 (47.9–58.9)	12.0 (0.0–27.5)
Euthanasia/death	0.4 (0.2–0.6)	0.2 (0.0–0.3)	0.2 (0.0–0.6)	35.4 (12.1–58.8)
OUTCOME				
Resolution (10 day)	72.6 (70.3–75.0)	73.9 (71.4–76.3)	67.7 (63.2–72.0)	29.5 (9.1–49.9)
Lost to follow-up	23.7 (21.4–26.0)	22.5 (20.2–24.9)	29.0 (24.7–33.2)	35.3 (13.8–56.8)

Longitudinal outcome is also displayed, with all comparisons shown when considered by consulting veterinary professional assessment of case severity at initial presentation.
[a] *Percentage of cases (95% confidence interval).*

TABLE 4 | Descriptive summary of cases where multiple sets of advice, nutraceutical dispensing, or pharmaceutical prescriptions were provided, expressed as a percentage of total cases where each "event type" was provided.

Event type		Percentage (%) of total advice/dispensing/prescription events, by event type						
	Total events	Diet change	Fast	Gastrointestinal nutraceutical	Systemic antimicrobial	Systemic anti-inflammatory	Gastrointestinal agent	Endoparasiticide/ endectocide
Diet change	2,227		14.0	64.3	49.0	13.4	37.0	7.7
Fast	608	52.0		54.1	50.0	22.2	43.8	5.1
Gastrointestinal nutraceutical	1,939	73.9	17.0		40.7	8.9	33.5	9.0
Systemic antimicrobial	1,585	68.9	19.0	49.8		18.2	37.2	6.0
Systemic anti-inflammatory	454	65.6	30.0	38.1	63.7		26.7	4.0
Gastrointestinal agent	1,200	68.6	22.0	54.2	49.2	10.1		4.2
Endoparasiticide/ endectocide	250	68.4	12.0	70.0	38.0	7.2	20.0	

For example, a gastrointestinal nutraceutical was also dispensed to 64.3% of cases where "diet change" advice was provided (n = 2,227).

Systemic Antimicrobial Prescription

Dogs presenting with diarrhoea with blood were more frequently prescribed a systemic antimicrobial (Odds ratio, OR, 4.1, 95% CI, 3.4–5.0), compared to diarrhoea without blood; moderate or severe cases were also more frequently prescribed a systemic antimicrobial compared to mild cases (OR 1.3, 95% CI 1.1–1.7) (**Table 5**). Compared to an interpreted normal or below 39°C body temperature at initial presentation, all other temperature categories were more frequently associated with prescription, peaking at between 39.5 and 39.9°C (OR 5.9, 95% CI 3.6–9.9). Prescription probability increased with age up to ∼7 years of age, but remained static between seven and thirteen, and increased from thirteen years of age upwards (**Figure 2A**). Results from univariable analyses are available in **Supplementary Table 4**. A cubic polynomial term was included to model age at consultation; no interaction terms significantly improved the fit of the model.

TABLE 5 | Parameter estimates from a finalised mixed effects logistic regression model, modelling on a case-level the presence of systemic antimicrobial and gastrointestinal agent prescription against a range of risk factors.

Random effect	Variance	SD[a]	Variable	Category	β	SE[b]	OR[c] (95% CI)[d]	*P*
SYSTEMIC ANTIMICROBIAL PRESCRIPTION								
Practice	0.75	0.87		Intercept	−0.72	0.14	0.49 (0.37–0.64)	
Site	0.27	0.52	Diarrhoea	Without blood	-	-	1.00	-
				With blood	**1.42**	**0.10**	**4.13 (3.42–4.98)**	**<0.01**
			Weight loss	Absent	-	-	1.00	-
				Present	0.71	0.40	2.03 (0.93–4.45)	0.08
			Severity	Mild	-	-	1.00	-
				Moderate/severe	**0.29**	**0.12**	**1.34 (1.06–1.69)**	**0.01**
			Diarrhoeic pattern	Large intestinal	-	-	1.00	-
				Mixed	0.01	0.13	1.01 (0.78–1.30)	0.95
				Small intestinal	0.07	0.11	1.07 (0.87–1.31)	0.54
				Unknown	**−0.53**	**0.17**	**0.59 (0.42–0.82)**	**<0.01**
			Body temperature	Normal/<39°C	-	-	1.00	-
				Not recorded	0.18	0.11	1.19 (0.97–1.46)	0.09
				39.0°C ≤ 39.4°C	**0.72**	**0.13**	**2.05 (1.58–2.65)**	**<0.01**
				39.5°C ≤ 39.9°C	**1.78**	**0.26**	**5.93 (3.56–9.88)**	**<0.01**
				40.0°C ≤	**1.50**	**0.48**	**4.47 (1.76–11.36)**	**<0.01**
			Age (years)	**Age—linear**	**0.22**	**0.08**	**1.25 (1.07–1.46)**	**0.01**
				Age—quadratic	**−0.25**	**0.08**	**0.78 (0.67–0.91)**	**<0.01**
				Age—cubic	**0.10**	**0.05**	**1.10 (1.01–1.20)**	**0.04**
GASTROINTESTINAL AGENT PRESCRIPTION								
Practice	0.32	0.56		Intercept	−1.65	0.13	0.19 (0.15–0.25)	
Site	0.28	0.53	Vomit	No vomit	-	-	1.00	-
				Without blood	**2.84**	**0.13**	**17.13 (13.41–21.89)**	**<0.01**
				With blood	**3.84**	**0.45**	**46.35 (19.39–110.81)**	**<0.01**
			Poor appetite	Absent	-	-	1.00	-
				Present	**0.65**	**0.15**	**1.92 (1.45–2.55)**	**<0.01**
			Severity	Mild	-	-	1.00	-
				Moderate & severe	**0.93**	**0.19**	**2.52 (1.76–3.62)**	**<0.01**
			Diarrhoeic pattern	Large intestinal	-	-	1.00	-
				Mixed	0.28	0.14	1.33 (1.00–1.76)	0.05
				Small intestinal	0.20	0.11	1.22 (0.97–1.53)	0.08
				Unknown	0.01	0.18	1.01 (0.70–1.44)	0.98
			Body temperature	Normal/<39°C	-	-	1.00	-
				Not recorded	**−0.38**	**0.12**	**0.68 (0.54–0.87)**	**<0.01**
				39.0°C ≤ 39.4°C	−0.04	0.15	0.96 (0.72–1.28)	0.78
				39.5°C ≤ 39.9°C	−0.17	0.25	0.84 (0.51–1.39)	0.50
				40.0°C ≤	**−1.05**	**0.50**	**0.35 (0.13–0.93)**	**0.04**
			Vomit & Severity	**No blood: moderate/severe**	**−0.71**	**0.25**	**0.49 (0.30–0.80)**	**0.01**
				With blood: moderate/severe	**-2.07**	**0.73**	**0.13 (0.03–0.53)**	**0.01**
			Age (years)	Age—linear	0.13	0.09	1.13 (0.95–1.35)	0.17
				Age—quadratic	**−0.33**	**0.09**	**0.72 (0.60–0.87)**	**<0.01**
				Age—cubic	0.09	0.05	1.10 (0.99–1.21)	0.08

Bold values are indicate significant findings.
[a]*Standard Deviation.*
[b]*Standard Error.*
[c]*Odds Ratio.*
[d]*95% Confidence Interval.*

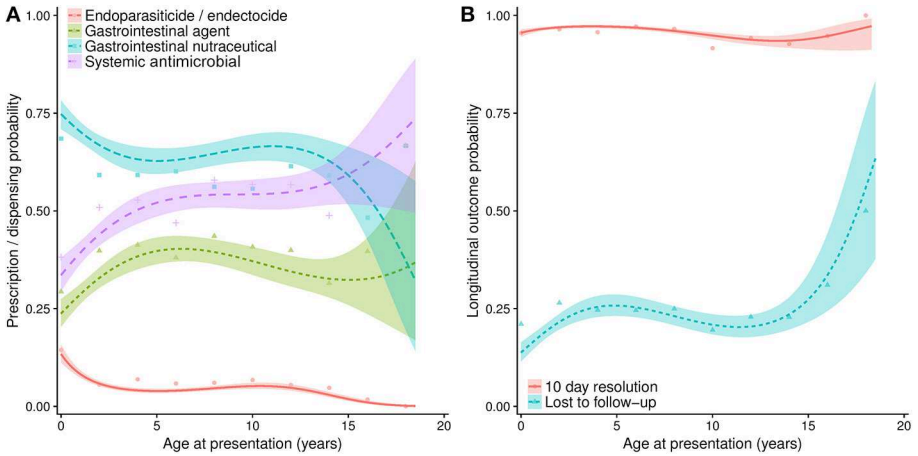

FIGURE 2 | (A) Projections from a series of multivariable logistic regression models, estimating the probability of a range of pharmaceutical agents being prescribed at initial presentation for diarrhoea, when considered against age at presentation (in years). **(B)** Estimates of longitudinal outcome, including probability of a case being lost to follow-up or being considered resolved of diarrhoeic clinical signs 10 days post-initial presentation. Lines refer to predicted probability, with shading relating to 95% confidence intervals to such predictions. Points are plotted to show original data points expressing the percentage of animals of each relevant age group (rounded to 2 year groups) that were prescribed a pharmaceutical agent, or were classified into the resolved or lost to follow-up categories.

Gastrointestinal Agent Prescription

Compared to non-vomiting dogs, dogs vomiting with or without blood were much more frequently prescribed a gastrointestinal agent (**Table 5**). Non-vomiting moderate and severe cases were also more frequently prescribed compared to non-vomiting mild cases (OR 2.5, 95% CI 1.8–3.6). Prescription probability increased up to approximately 6 years of age, before decreasing until 15 years of age (**Figure 2A**). Univariable results are available in **Supplementary Table 5**. A cubic polynomial term was included to model age at consultation; an interaction term between case severity and vomiting significantly improved the fit of the model.

Endoparasiticide/Endectocide Prescription

Animals reported to have lost weight were associated with increased odds (OR 3.4, 95% CI 1.3–9.0) of endoparasiticide and/or endectocide prescription, though moderate and severe cases (OR 0.4, 95% CI 0.3–0.7) or vomiting cases without blood (OR 0.5, 95% CI 0.4–0.7) were less frequently prescribed (**Table 6**). Vaccinated animals were also less frequently (OR 0.6, 95% CI 0.4–0.7) prescribed at initial presentation. Prescription probability decreased sharply up to 3 years of age, remained broadly stable until 12 years of age, and then decreased further (**Figure 2A**). Univariable results are available in **Supplementary Table 6**. A cubic polynomial term was included to model age at consultation; no interaction terms significantly improved the fit of the model.

Dispensing of Gastrointestinal Nutraceuticals

A number of clinical signs including diarrhoea with blood (OR 0.7, 95% CI 0.6–0.9), vomiting with (OR 0.3, 95% CI 0.2–0.5) or without blood (OR 0.6, 95% CI 0.5–0.7), body temperature between 39.5 and 39.9°C (OR 0.5, 95% CI 0.3–0.8), other clinical signs (OR 0.4, 95% CI 0.2–0.7), and moderate and severe cases (OR 0.6, 95% CI 0.5–0.8) were all less frequently associated with a gastrointestinal nutraceutical being dispensed (**Table 6**). However, a mixed diarrhoeic pattern was associated with increased odds (OR 1.33, 95% CI 1.04–1.71). Odds decreased with age to approximately 4 years of age and remained broadly static until 10 years of age, before decreasing further (**Figure 2A**). Univariable results are available in **Supplementary Table 7**. A cubic polynomial term was included to model age at consultation; no interaction terms significantly improved the fit of the model.

Analysis of Longitudinal Outcomes

Cases Lost to Follow-Up

In total, 754 cases (23.6% of total cases) were lost to follow-up. Currently insured (OR 0.7, 95% CI 0.5–0.9), recently vaccinated (OR 0.3. 95% CI 0.3–0.4) or neutered (OR 0.4, 95% CI 0.3–0.5) dogs had lower odds of being lost to follow-up (**Table 7**). Increasing age was associated with increased probability of a case being lost to follow-up until approximately 4 years of age, decreasing slightly between four and twelve, before increasing once more (**Figure 2B**). Univariable results are available in **Supplementary Table 8**. A cubic polynomial term was included to model age at consultation; no interaction terms significantly improved the fit of the model.

Diarrhoea Resolution

Cases euthanised on initial presentation ($n = 6$) and lost to follow-up ($n = 754$) were excluded, leaving 2,429 cases available for resolution analyses. By the 10th day following initial presentation, 95.4% (95% CI 94.5–96.3) of cases were considered resolved; 7.6% of resolved cases were recorded as such in the clinical narrative, the remaining cases were assumed to be resolved by the absence of any further gastrointestinal-related consultations between 11 and 30 days following initial presentation.

TABLE 6 | Parameter estimates from a finalised mixed effects logistic regression model, modelling on a case-level the presence of endoparasiticide/endectocide prescription and dispensing of gastrointestinal nutraceuticals against a range of risk factors.

Random effect	Variance	SD[a]	Variable	Category	β	SE[b]	OR[c] (95% CI)[d]	*P*
ENDOPARASITICIDE/ENDECTOCIDE PRESCRIPTION								
Practice	0.28	0.53		Intercept	−2.38	0.19	0.09 (0.06–0.14)	
Site	0.21	0.46	Vomit	No vomit	-	-	1.0	-
				Without blood	**−0.69**	**0.18**	**0.50 (0.36–0.71)**	**<0.01**
				With blood	−1.04	0.74	0.35 (0.08–1.50)	0.16
			Weight loss	Absent	-	-	1.0	-
				Present	**1.22**	**0.50**	**3.37 (1.27–9.00)**	**0.02**
			Severity	Mild	-	-	1.0	-
				Moderate & severe	**−0.83**	**0.27**	**0.44 (0.26–0.74)**	**<0.01**
			Vaccination status	Unvaccinated	-	-	1.0	-
				Vaccinated	**−0.59**	**0.15**	**0.55 (0.42–0.74)**	**<0.01**
			Age (years)	Age—linear	0.07	0.18	1.07 (0.76–1.51)	0.70
				Age—quadratic	**0.57**	**0.14**	**1.77 (1.35–2.32)**	**<0.01**
				Age—cubic	**−0.36**	**0.11**	**0.70 (0.57–0.87)**	**<0.01**
GASTROINTESTINAL NUTRACEUTICAL DISPENSING								
Practice	0.64	0.80		Intercept	0.93	0.16	2.52 (1.85–3.43)	
Site	0.21	0.45	Diarrhoea	Without blood	-	-	1.0	-
				With blood	**−0.31**	**0.09**	**0.74 (0.62–0.88)**	**<0.01**
			Vomit	No vomit	-	-	1.0	-
				Without blood	**−0.57**	**0.09**	**0.57 (0.47–0.68)**	**<0.01**
				With blood	**−1.34**	**0.29**	**0.26 (0.15–0.46)**	**<0.01**
			Other signs	Absent	-	-	1.0	-
				Present	**−0.99**	**0.33**	**0.37 (0.19–0.71)**	**<0.01**
			Severity	Mild	-	-	1.0	-
				Moderate & severe	**−0.48**	**0.12**	**0.62 (0.49–0.78)**	**<0.01**
			Diarrhoeic pattern	Large intestinal	-	-	1.0	-
				Mixed	**0.29**	**0.13**	**1.33 (1.04–1.71)**	**0.02**
				Small intestinal	0.21	0.11	1.23 (1.00–1.51)	0.05
				Unknown	−0.20	0.16	0.82 (0.60–1.12)	0.22
			Body temperature	Normal/<39°C	-	-	1.0	-
				Not recorded	**−0.50**	**0.10**	**0.61 (0.50–0.74)**	**<0.01**
				39.0°C ≤ 39.4°C	−0.15	0.13	0.86 (0.67–1.11)	0.25
				39.5°C ≤ 39.9°C	**−0.66**	**0.22**	**0.52 (0.34–0.80)**	**<0.01**
				40.0°C ≤	−0.57	0.42	0.57 (0.25–1.28)	0.17
			Vaccination status	Unvaccinated	-	-	1.0	-
				Vaccinated	0.15	0.10	1.16 (0.96–1.40)	0.13
			Age (years)	Age—linear	0.03	0.08	1.03 (0.89–1.20)	0.70
				Age—quadratic	**0.26**	**0.08**	**1.29 (1.11–1.51)**	**<0.01**
				Age—cubic	**−0.14**	**0.05**	**0.87 (0.80–0.95)**	**<0.01**

Bold values are indicate significant findings.
[a]*Standard Deviation.*
[b]*Standard Error.*
[c]*Odds Ratio.*
[d]*95% Confidence Interval.*

Univariable analyses are available in **Supplementary Table 9**. Dogs presenting with a mixed (OR 0.57, 95% CI 0.33–0.98) or unknown (OR 0.46, 95% CI 0.25–0.85) diarrhoeic pattern were less frequently resolved (**Table 7**). Though no pharmaceutical agent prescribed exclusively were associated with significant variant odds of resolution, when owners were provided with dietary modification advice combined with gastrointestinal nutraceuticals but no other therapy, such cases had increased odds of resolution by 10 days post initial presentation (OR 2.8, 95% CI 1.3–6.1). This latter finding was also observed when only mild, normothermic (<39.5°C), non-haemorrhagic cases were modelled (data not presented). There was little variation in probability of resolution and age (**Figure 2B**). A cubic polynomial term was included to model age at consultation;

TABLE 7 | Parameter estimates from a finalised mixed effects logistic regression model, modelling on a case-level loss to follow-up and 10 day diarrhoea resolution against a range of risk factors.

Random effect	Variance	SD[a]	Variable	Category	β	SE[b]	OR[c] (95% CI)[d]	*P*
LOSS TO FOLLOW-UP								
Practice	0.42	0.65		Intercept	0.29	0.16	1.34 (0.98–1.82)	
Site	0.24	0.49	Diarrhoea	Without blood	-	-	1.0	-
				With blood	0.15	0.10	1.16 (0.96–1.40)	0.13
			Insurance status	Uninsured	-	-	1.0	-
				Insured	**−0.36**	**0.13**	**0.70 (0.54–0.89)**	**<0.01**
			Neutered status	Unneutered	-	-	1.0	-
				Neutered	**−0.88**	**0.10**	**0.41 (0.34–0.51)**	**<0.01**
			Vaccination status	Unvaccinated	-	-	1.0	-
				Vaccinated	**−1.16**	**0.10**	**0.32 (0.26–0.39)**	**<0.01**
			Gastrointestinal agent	Not prescribed	-	-	1.0	-
				Prescribed	**0.32**	**0.10**	**1.38 (1.14–1.66)**	**<0.01**
			Age (years)	Age—linear	−0.09	0.09	0.92 (0.77–1.10)	0.35
				Age—quadratic	**−0.38**	**0.09**	**0.69 (0.58–0.82)**	**<0.01**
				Age—cubic	**0.20**	**0.05**	**1.22 (1.11–1.35)**	**<0.01**
DIARRHOEA RESOLUTION								
Practice	0.04	0.19		Intercept	3.50	0.29	33.00 (18.50–58.80)	
Site	0.07	0.26	Vomit	No vomit	-	-	1.0	-
				Without blood	**0.45**	**0.23**	**1.58 (1.01–2.46)**	**0.05**
				With blood	−0.87	0.47	0.42 (0.17–1.05)	0.06
			Diarrhoeic pattern	Large intestinal	-	-	1.0	-
				Mixed	**−0.57**	**0.28**	**0.57 (0.33–0.98)**	**0.04**
				Small intestinal	−0.25	0.24	0.78 (0.48–1.25)	0.30
				Unknown	**−0.78**	**0.32**	**0.46 (0.25–0.85)**	**0.01**
			Diet advice + GI nutraceutical alone	Not dispensed	-	-	1.0	-
				Dispensed	**1.03**	**0.40**	**2.79 (1.27–6.12)**	**0.01**
			Age (years)	Age—linear	−0.33	0.19	0.72 (0.50–1.04)	0.08
				Age—quadratic	**−0.44**	**0.19**	**0.65 (0.44–0.94)**	**0.02**
				Age—cubic	0.20	0.11	1.22 (0.98–1.52)	0.08

Bold values are indicate significant findings.
[a]*Standard Deviation.*
[b]*Standard Error.*
[c]*Odds Ratio.*
[d]*95% Confidence Interval.*

no interaction terms significantly improved the fit of the model.

DISCUSSION

Canine acute diarrhoea is a frequent cause of presentation to primary veterinary practice (5); a range of aetiologies are associated with diarrhoea (6), a minority of which can be life threatening (9). When cases are first presented, practitioners need to make complex decisions around case management, often in the absence of any specific diagnosis (5). There is a need to understand these choices and to explore new ways of evidencing their effect, particularly in the context of systemic antimicrobial prescription. Here we used EHRs collected from a large number of veterinary practices, supplemented by structured questionnaire responses, to describe clinical signs exhibited by dogs with acute diarrhoea, characterise common management and treatment strategies, and assess the outcome of cases observed longitudinally.

This study represented the first attempt to harness overall veterinary-assessed opinion of case severity, with the vast majority being described as mild (83.6%). Only 17 cases were classed as severe, with six of these being euthanised on initial presentation. Whilst this limited our ability to describe severe disease, our findings further confirmed diarrhoea as primarily a mild condition in dogs (1). In this study, the majority of cases presented with non-vomiting, non-haemorrhagic diarrhoea, broadly consistent with previous studies (2, 5). However, diarrhoea with blood (41% of cases) and vomiting (36% of cases) was more common than previously described (25 and 18%, respectively) (5). This previous study considered all cases of diarrhoea regardless of clinical sign duration, also observing

"uncomplicated diarrhea" (absence of vomiting or haemorrhagic diarrhoea) to be more common in cases of longer disease duration. This suggests that the presence of clinical signs potentially alarming to owners' e.g., haemorrhagic diarrhoea, might prompt these owners to seek veterinary attention more rapidly, potentially explaining the higher prevalence of such signs recorded here in acute cases.

Diagnostic tests were rarely used in this population (9% of all cases), and less commonly than previously reported (3, 5). This might again reflect the primary presentation nature of this study, and the generally mild nature of the reported disease. Hence, it can be reasonably assumed that most prescriptions described in this population were empirical, particularly considering that the majority were provided at initial presentation rather than over the following 10 days. Medical prescribers often perceive pressure to implement a material management plan (30) which may lead to unnecessary prescriptions, including those for antimicrobials (31); it is possible that such pressures might also influence veterinary prescription decisions (32). Diagnostic investigation should take place if an infectious aetiology is suspected (33). Unfortunately, it was not possible to determine the number of cases where the consulting veterinary professional suspected such an infectious aetiology in this study; such analyses could be of considerable future value, particularly in relation to antimicrobial stewardship.

Presence of blood in diarrhoea was significantly associated with increased odds of a systemic antimicrobial prescription being provided. This has been previously observed (5), and likely reflects a perception of increased bacteraemia risk (33). However, there is increasing evidence to suggest antimicrobial therapy is not required in such cases (9, 10, 15, 23), with a recent study finding a significant proportion of canine AHDS patients fulfilling clinical bacteraemia criteria actually tested negative on blood culture (9). Odds of a systemic antimicrobial prescription were also increased for all body temperature categories exceeding 39.0°C. Of note, body temperature was inconsistently recorded, revealing a limitation of clinical narrative analyses. Nevertheless, our findings suggest differences of opinion as to what body temperature would indicate presence or high risk of bacteraemia. Although pyrexia has been defined as body temperature in excess of 39.7°C (34), previous studies focusing on diarrhoea have variably defined pyrexia/hyperthermia between 38.8 and 39.5°C (2, 22), even altering definition by dog size (9). This study identified that 35.7% of normothermic (under 39.5°C), mild, non-haemorrhagic cases (n = 1,050) still prescribed systemic antimicrobials at initial presentation. On this evidence, it would thus seem that our study has identified a reasonable proportion of cases not at clear risk of sepsis treated with systemic antimicrobials regardless, in contravention to current prescribing guidance[5]. Hence, establishing a consistent definition of sepsis risk may be of some importance for effective antimicrobial stewardship. Assisting practitioner identification of patients at risk of sepsis remains a challenge across veterinary and medical care (35). In the absence of a specific diagnosis, clinical scoring has previously been successfully utilised to uniformly measure clinical severity and response to therapy (9, 10, 22). It could be of value to define more universal indicators of sepsis, and to investigate the potential benefit which could be gained, both epidemiologically and practically, from routinely applying such methods in first opinion practice.

The most frequently prescribed systemic antimicrobial in this study was metronidazole, consistent with previous studies (5, 21). This finding also suggests that the predominant concern of the prescribing veterinary surgeon is treatment of anaerobic bacterial species e.g., *Clostridium perfringens*, though the causative role of such bacteria in gastrointestinal disease has recently been brought into question (16). Further, current prescribing guidance recommends metronidazole use for chronic diarrhoea/chronic enteropathy treatment trials alone once all other diagnostic test and empirical treatment options have been exhausted (36), again suggesting limited compliance with existing guidance. In total, systemic antimicrobials were prescribed to 50% of cases, comparable or lower than previously described (46.5, 63, and 71%) (2, 5, 7). We have recently identified an approximately 30% reduction in systemic antimicrobial prescription in consultations for gastrointestinal disease between 2014 and 2018 (and a simultaneous approximately 25% increase in gastrointestinal nutraceutical prescription frequency) (37). Though it was not possible to observe a direct change in management approach by individual veterinary surgeons as repeated measures per surgeon were not recorded, our findings here could suggest that the manner with which veterinary surgeons manage gastroenteric disease and acute canine diarrhoea is changing. However, a prospective cohort study might be better placed to demonstrate this more definitively. If present, this finding might reflect increased awareness of voluntary prescribing guidance recommending antimicrobial therapy to be reserved only for acute diarrhoea cases exhibiting, or at risk, of bacteraemia or sepsis. We further recognise the opportunities afforded by providing prescription benchmarking statistics to practitioners, enabling them to effectively reflect on their own decision-making and consider changing as a result. Indeed this is an area of active development for us currently through projects such as "mySavsnetAMR;" all practices participating in SAVSNet also enjoy free access to a secure, anonymised benchmarking website for this purpose (38).

In contrast to systemic antimicrobial prescription, gastrointestinal nutraceutical dispensing frequency was considerably greater than in a previous study (61% of cases compared to 26%) (2). Study methodological differences accepted, it has been suggested that gastroenteric nutraceuticals may form a "no harm" alternative to antibiosis in order to effectively manage owner treatment plan expectations (32). Of further interest, our findings suggest that a combination of dietary modification and gastrointestinal nutraceuticals without prescription of any studied pharmaceutical agent could aid resolution of diarrhoeic clinical signs. Though evidence remains scarce, previous studies have suggested that probiotics might be efficacious in ameliorating infectious, non-infectious or idiopathic diarrhoea in dogs (25); it is possible that we might be observing such an effect here. However, since we have

[5]PROTECT ME. Available from: https://www.bsava.com/Resources/Veterinary-resources/PROTECT-ME

not randomised cases into treatment groups, there remains a possibility of bias according to over-simplification of clinical severity scoring as used here (39). Therefore, whilst evidence of *in vivo* efficacy of gastrointestinal nutraceuticals remains limited (25) we advocate some continued caution over wholeheartedly embracing nutraceutical use. As with all other areas of veterinary practice, clear clinical evidence when available should drive decision making, and when unavailable efforts should be made to fill such gaps in knowledge. As such, we believe the field is now ready for a fully randomised pragmatic trial to provide more definitive evidence surrounding the clinical benefit (or absence thereof) of prescribing antimicrobials and other agents to manage acute canine diarrhoea.

Regarding endoparasiticides/endectocides, weight loss was significantly associated with increased odds of prescription, possibly reflecting the view that weight loss is often associated with parasitic infection (33). It should be remembered that some endoparasiticides/endectocides[6] (as well as gastrointestinal nutraceuticals) are available without the need of a prescription such that it is likely we have under-estimated the actual use of these agents in this study. Similarly, this study focused on in-consultation prescription decisions; expanding its scope to include EHRs for referred animals and in-patient (hospitalised) records would more completely represent all aspects of companion animal practice.

The effect of the animal's age on odds of pharmaceutical prescription were complex and could be separated into two groups: systemic antimicrobials or gastrointestinal agents were prescribed more commonly to older animals, whereas endoparasiticides/endectocides or gastrointestinal nutraceuticals were prescribed more commonly to younger animals, possibly reflecting increased parasitic or viral infection in puppies (33, 40). On univariable analyses, odds of a case being considered moderate or severe did increase with age (data not presented); however, including severity as an interaction with age did not improve the fit of the model.

Classical approaches to defining the benefit of particular treatments is to use randomised control trials, systematic reviews or meta-analyses. For canine gastroenteritis treated in primary care, trials of any form whether randomised or not are limited in number, and generally small in size (9, 10, 22), such that there is a dearth of evidence with which practitioners can base their treatment choices. One route to increasing evidence and complementing the highest level data from trials are pragmatic and observational studies using EHRs collected at scale (41). Here, our observational approach suggests no clear link between any therapy choice and outcome; a finding corroborated for antimicrobial therapy by earlier smaller studies (9, 10, 22). There is an increasing pressure on both medical and veterinary prescribers to make responsible therapeutic decisions, reflecting best available clinical evidence (19), and our findings would appear to broadly support the view that using antimicrobials for management of acute diarrhoea is largely unnecessary (10).

[6]Veterinary Medicines Directorate product information database. Available from: www.vmd.defra.gov.uk/ProductInformationDatabase/Default.aspx

Whilst the purely observational approach used here was useful, this study was limited by nearly a quarter of cases being lost to follow-up. Without specific intervention, it is impossible to determine whether these cases simply recovered, moved to another veterinary practice, opted out of further SAVSNET participation, or died. However, we did show insured, neutered, or vaccinated dogs to be associated with significantly decreased odds of being lost to follow-up, suggesting either owners of such dogs are more likely to engage with regular veterinary care, or their vets are more likely to request follow up consultations. Similarly, the odds of being lost to follow-up also broadly increased as an animal's age increased. Whilst this might represent increased odds of death (42), it might also suggest that as owners become more experienced with their pet, they are less likely to re-present with their dog when investigating/treating disease.

Defining outcome in an observational study of this type presents certain challenges. When reviewing cases that re-presented within 10 days of initial presentation, dogs often re-presented at the request of the veterinary surgeon, or re-presented for an unrelated complaint (data not presented). We therefore concluded that time between initial and subsequent presentations alone to be an unreliable measure of clinical resolution and response to treatment. Thus, we used a 10 day period as a broad representation of the acute diarrhoea therapy period (9, 10, 22, 33), subsequently using MPC between 11 and 30 days as an indicator of gastroenteric clinical sign persistence or re-emergence. Though seemingly appropriate, loss to follow-up limited our ability to fully characterise clinical resolution. In addition, considerable therapeutic management diversity was seen; this represents a significant challenge when seeking to define the effect of each pharmaceutical intervention which would only be compounded if we had also considered additional factors such as dosage or course length. Here we focused on five pharmaceutical classes commonly prescribed to diarrhoea cases (2); other pharmaceutical classes were prescribed which might also have had an impact on clinical resolution. Considering these limiting factors, a more structured approach, including contacting owners after initial presentation, could complement the more observational approach taken here.

The issues posed by veterinary surgeons failing to record, or variably recording information within the clinical narrative has been previously noted (7). We also observed such difficulties (e.g., body temperature recording), though we found combining compulsory randomised questionnaire data with the EHR to at least partially mitigate this issue (e.g., case severity). To encourage engagement, the questionnaire was only deployed in a small proportion of randomly selected relevant consultations; the cases studied here therefore only represent a small percentage of cases available within the SAVSNET database. It should also be remembered that questionnaire responses were self-defined; individual variation in case definition is therefore possible. As text mining capabilities advance (7), the confidence with which we could identify and follow cases using such approaches is likely to increase, potentially unlocking a considerably greater number of cases for analyses. However, in the mean time we

would advocate the use of a combined textual and questionnaire response analysis as demonstrated here.

CONCLUSIONS

This study successfully demonstrated the ability of combined structured, semi-structured and unstructured data to characterise factors associated with pharmaceutical prescription in acute canine diarrhoea cases. Not surprisingly, we saw considerable therapeutic diversity between cases, a number of which contradicted current prescribing guidance. Considering the threat posed by antimicrobial resistance especially, this suggests that latest clinical evidence is not effectively being disseminated throughout the profession. The findings presented here complement other studies, and suggests that efforts should be re-doubled to effectively disseminate latest clinical evidence to the wider, and particularly first opinion, veterinary profession. Though future methodological improvements are recommended, this study broadly supports the view that systemic antimicrobials are largely unnecessary in acute diarrhoea cases. The only intervention positively associated with resolution odds was provision of dietary modification advice and gastrointestinal nutraceuticals; hence we would urgently recommend further work exploring the precise impact of prebiotics, probiotics etc. on gastrointestinal health in our canine population.

DATA AVAILABILITY

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

DS devised study design, analysed data, and drafted the manuscript, supervised by PJ, PN, GP, and AR. NW is a co-investigator on the grant funding this work, and assisted with revising the manuscript. FS-V and SD are co-investigators of the wider SAVSNET project, assisting with revising the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2019.00218/full#supplementary-material

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METHODS
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Developing Canadian Defined Daily Doses for Animals: A Metric to Quantify Antimicrobial Use

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Antimicrobial use surveillance data need to be analyzed and reported in a standardized and harmonized way. In veterinary medicine, one approach is to use defined daily doses (DDD) for animals. DDD for animals are technical standards used in various measures or metrics that quantify antimicrobial use. The European Medicines Agency published principles for assigning DDDvet values based on information on dosing obtained from nine European countries. For measuring antimicrobial use in livestock within Canada, DDDs for animals reflective of Canadian veterinary antimicrobial use (DDDvetCAs) were needed. Our objectives were (1) to describe the development of DDDvetCA standards for pigs and poultry (broiler chickens and turkeys) for authorized and compounded antimicrobial active ingredients used in Canada, including those used extra-label; and (2) to compare the DDDvetCAs with EMA's DDDvets, where possible. Species-specific DDDvetCAs were assigned based on the average of unique antimicrobial daily doses obtained from product information, stratified by route of administration and age indication (where applicable). The feed, water and bolus DDDvetCAs were compared to oral DDDvets, and injectable DDDvetCAs to parenteral DDDvets, that matched by antimicrobial active ingredient. Seventy-five DDDvetCAs were assigned for pigs; 51 for poultry. Seventeen injectable DDDvetCAs could be compared to 14 EMA's parenteral DDDvets and 53 feed, water, and bolus DDDvetCAs could be compared to 40 oral DDDvets. Feed and water DDDvetCAs were generally lower than EMA's oral DDDvets, although differences in methodology between Canada and Europe make comparisons challenging. The assignment of DDDvetCAs was a resource intensive and iterative process. EMA's published principles for assigning DDDvets were an invaluable source of information. The use of DDDvetCAs will reflect exposure of Canadian animals to antimicrobials, be useful for evaluating associations between use and resistance within Canada and provide information for risk assessment and stewardship policies. However, when reporting antimicrobial use data internationally, using the same DDD standards as other reporting countries will facilitate between country comparisons, although differences in which antimicrobial active ingredients are licensed between countries may create challenges. Future steps include assigning DDDvetCAs for other food animal species, such as cattle, veal, and farmed fish.

Keywords: DDDvet, antimicrobial usage, veterinary, Canada, surveillance

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INTRODUCTION

Antimicrobials have an important role in food animal production. Their use to treat, control, and prevent infections plays a part in the sustainability of food animal production (1). However, antimicrobial use (AMU), in both humans and animals, has led to the emergence of antimicrobial resistance (AMR), with a subsequent increased incidence of infections that are more difficult to treat (2). These infections have significant impacts on humans, with an estimated 700,000 people globally dying every year of drug-resistant infections in the world (2), and likely a significant impact on animals, though this information is not often reported.

For these reasons, some countries conduct surveillance of antimicrobials used in animals (3–5). The Public Health Agency of Canada's Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) conducts AMU surveillance in food animals (5). These surveillance activities align with various national and international initiatives and action plans to address the threat of AMR (6–8). CIPARS currently reports information provided by the Canadian Animal Health Institute on the quantities of antimicrobial agents distributed for use in animals. For 2018 data, CIPARS will be reporting on antimicrobials sold for use in animals. This data, collected under new legislative authority, will be provided by pharmaceutical manufacturers, importers, and compounders. At the farm level, CIPARS currently collects information on AMU and AMR in grower-finisher pigs, broiler chickens, and turkeys, with the aim to expand surveillance into other food animal sectors (5). This information is used to fulfill the objectives of the CIPARS farm surveillance component which are to monitor trends in antimicrobial use in select species of livestock (5).

Data gathered by AMU surveillance programs must be analyzed and reported in a standardized and harmonized way to draw conclusions that are as accurate as possible. In addition to monitoring trends in AMU, these data are needed to develop effective farm and veterinary interventions, inform antimicrobial stewardship, and to provide information for risk assessment.

One approach for animal AMU analysis and reporting is to apply a defined daily dose (DDD) for animals. The DDD for animals is a technical unit of measurement developed by the European Medicines Agency's (EMA) European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) project (9). ESVAC coined the term DDDvet to describe their DDD for animals, which are used in various metrics to quantify AMU (9). The DDD are used to adjust the kilograms of active antimicrobial ingredients (AAIs) by the daily dose of the antimicrobial, measured in mg per kilogram of animal (9). This concept is based on the globally accepted DDD in human medicine (10).

Abbreviations: AMU, antimicrobial use; AMR, antimicrobial resistance; AAI, active antimicrobial ingredient; CIPARS, Canadian Integrated Program for Antimicrobial Resistance Surveillance; DDD, defined daily dose; DDD for animals, defined daily doses for animals; DDDvetCA, Canadian defined daily doses for animals; DDDvet, European Medicines Agency defined daily doses for animals; EMA, European Medicines Agency; ESVAC, European Surveillance of Veterinary Antimicrobial Consumption.

The creation (or assignment) of standardized DDD for animals involves determining an average dose for each AAI authorized for use in the species of interest by route of administration (9). The principles of DDD assignment may also be extended to AAIs authorized for use in another species and used in an extra-label manner in the species of interest. As technical standards, these assigned DDDs are not meant to be considered recommended doses and may not represent doses that are used in practice (9). Instead, the assigned DDDs simply provide standard doses that can be used to facilitate standardized measurements of AMU. These standardized measurements can be used to examine trends in AMU over time, to compare of AMU between different regions, across species, and different AAIs, and to examine associations between AMU and AMR (9).

Accounting for dose when analyzing and reporting AMU is important since dosing between antimicrobials varies. This variation in dose may be due to differences in mechanism of action, pharmaceutical formulations, and metabolism and distribution in the body (11). To demonstrate the importance of accounting for dose when comparing antimicrobial use on two farms, we have provided the following hypothetical example. During one production cycle, farm A gives a single injection of ceftiofur to 100 grower-finisher pigs at 3 mg/kg, and farm B gives a single injection of tiamulin to 100 grower-finisher pigs at 11 mg/kg. Both farms have 1,000 grower-finisher pigs (with an average weight at treatment of 65 kg). Farm A used 19,500 mg of ceftiofur, which equals 0.3 mg per kg of animal, while farm B used 71,500 mg of tiamulin, equal to 1.1 mg/kg of animal. From a weight perspective, farm A appears to have used less antimicrobial than farm B, yet each farmer administered the same number of treatments to the same number of animals. If we adjust the kilograms of antimicrobial used by the dose, we find that both farm A and B used 6,500 DDD for animals (kg). Another way to interpret this value is say that both farms treated 6,500 kg of pig with one daily dose of antimicrobial. By adjusting the weight of the antimicrobial given by its DDD for animals, comparisons in use between antimicrobials with different doses are more informative.

In 2015, the EMA published principles for assigning DDDvets (9) with the goal to harmonize where possible with the methodology published by the World Health Organization (10). EMA's published principles for assigning DDDvets were invaluable in informing and guiding this project (9). These principles for assigning DDDvets were followed in 2016 by the publication of EMA assigned DDDvets for pigs, cattle and poultry, based on product information on dosing for veterinary antimicrobials obtained from Summaries of Product Characteristics (SPC) from nine European countries (12). Prior to the publication of DDDvets by the EMA, Postma et al. (13) had described assigning defined daily dose animal (DDDA) for each antimicrobial product authorized for use in pigs, using product information from four European countries. Other countries have developed national DDD for animals, including the Netherlands and Denmark, although different terminology is used to describe them (14, 15).

Using the principles for assigning DDDvets published by EMA, CIPARS decided to develop Canadian DDD for animals

(DDDvetCAs). The decision to develop DDDvetCAs was made because of expected differences in antimicrobials between Canada and other countries, including antimicrobials registered for use, antimicrobial doses, the number of unique doses for an antimicrobial, and indications for use. Standardized DDD for animals based on antimicrobials authorized for use in Canada were needed for the analysis of AMU within a Canadian context. The primary objective of this study was to develop DDDvetCAs for all antimicrobials authorized (or otherwise known to be used in an extra-label manner) for use in Canada, starting with pigs and poultry (including broiler chickens and turkeys). A secondary objective was to compare DDDvetCAs with EMA's DDDvets for both species.

METHODS

We used the EMA's *Principles for the assignment of defined daily doses for animals* as guidance in the assignment of DDDvetCAs for pigs and poultry, with minor changes as required (9).

Collection of Antimicrobial Daily Dose Information

Decisions about which antimicrobials and which doses to include in the assignment of DDDvetCAs differed in some ways to decisions made by EMA. In contrast to EMA, we assigned DDDvetCAs to coccidiostats and ionophores, as farm surveillance data about their use are collected and they are classified in Canada as antimicrobials. Also included were antimicrobials with growth promotion properties, such as bacitracin, virginiamycin, and avilamycin. Compounded antimicrobials with no equivalent authorized product in the species of interest and those used in an extra-label manner were also included, with evidence of use from Canadian surveillance data. Extra-label drug use was defined as use of an antimicrobial in a species or by route of administration not described on the label.

Daily doses from antimicrobial products authorized for use in Canada were obtained from product information found in the Canadian Compendium of Veterinary Products (16) and the Compendium of Medicating Ingredient Brochures (17). Information on doses for compounded antimicrobials were obtained from a survey in one province, that collected the prescribed dose from the label applied to the product by the veterinarian (Cécile Ferrouillet, personal communication, 2017). Doses for antimicrobials used in an extra-label manner were obtained from expert opinion (Agnes Agunos, personal communication, 2016; Anne Deckert, personal communication, 2016).

Microsoft Excel® (2010) was used to tabulate the unique daily doses for each AAI, regardless of indication. Doses were stratified by species and route of administration (in feed, in water, by injection, and by individual oral treatment or bolus). While EMA chose to group oral routes of administration together, we chose to keep them in separate categories. Since product information for feed and water medications often include doses in units such as mg/kg feed or mg/L water, conversion to mg/kg body weight was required. To do so, these doses were multiplied by either the feed or water to weight conversion ratio (in kg feed per kg animal or in L water per kg animal), as per ESVAC (9). Also, in most cases, only treatment and prevention doses were tabulated; growth promotion doses were excluded, except where the only doses available for an antimicrobial were for growth promotion purposes. This exception applied to most coccidiostats and ionophores in pigs, and to bambermycin and benzylpenicillin used in feed in poultry. These doses were clearly labeled as growth promotion doses in order to clearly identify where they were used.

Due to heterogeneity in drug product information, decisions had to be made during the tabulation of product doses (**Figure 1**). If the product information recommended an initial loading dose followed by a maintenance dose, the daily dose was determined by calculating the total dose given over the recommended number of days of treatment, divided by the recommended number of days. For combination products containing more than one AAI, the daily dose of each AAI in the combination was determined by multiplying the dose of the combination product by the proportion of each AAI in the product. If a dose range was reported, the mean of the range was used to assign the DDDvetCA. If the dose was expressed in international units, the dose was converted to mg with conversion factors used by the EMA (18). To obtain a daily dose for long-acting injectable products, the dose was divided by either the duration of action (in days) if available, or the recommended dosing frequency (e.g., every 3 days) if the duration of action was not available. In some instances, we contacted product manufacturers to get information about durations of action when the product information was unclear (i.e., tulathromycin, and benzathine benzylpenicillin, and procaine benzylpenicillin). When the concentration of an AAI was reported as both a salt and a base, the salt concentration was used to calculate the daily dose. For example, an in-feed product containing tiamulin for use in pigs indicated that 1 kg of product contained 100 g of tiamulin hydrogen fumarate (the salt), equaling 80.9 g of tiamulin base. In this case, the salt concentration of 100 g/kg was used to calculate the daily dose.

The distribution of doses was examined by calculating the minimum, maximum, and median daily doses for each AAI, in addition to the mean daily dose. To compare the mean daily dose (DDDvetCA) to the median dose, a ratio was calculated using Equation (1).

$$Ratio\ mean : median\ dose = DDDvetCA / median\ dose \quad (1)$$

Additional Information Recorded

In addition to dose information, the Health Canada Category of the AAI was recorded (19). These categories include Category I—Very high importance (to human medicine), Category II—High importance, Category III—Medium importance and Category IV—Low importance (19). These categories are distinguished by their importance in human medicine and the availability of effective alternatives should resistance occur. Health Canada category I and II antimicrobials are used to treat serious infections in humans, however, if resistance to category II

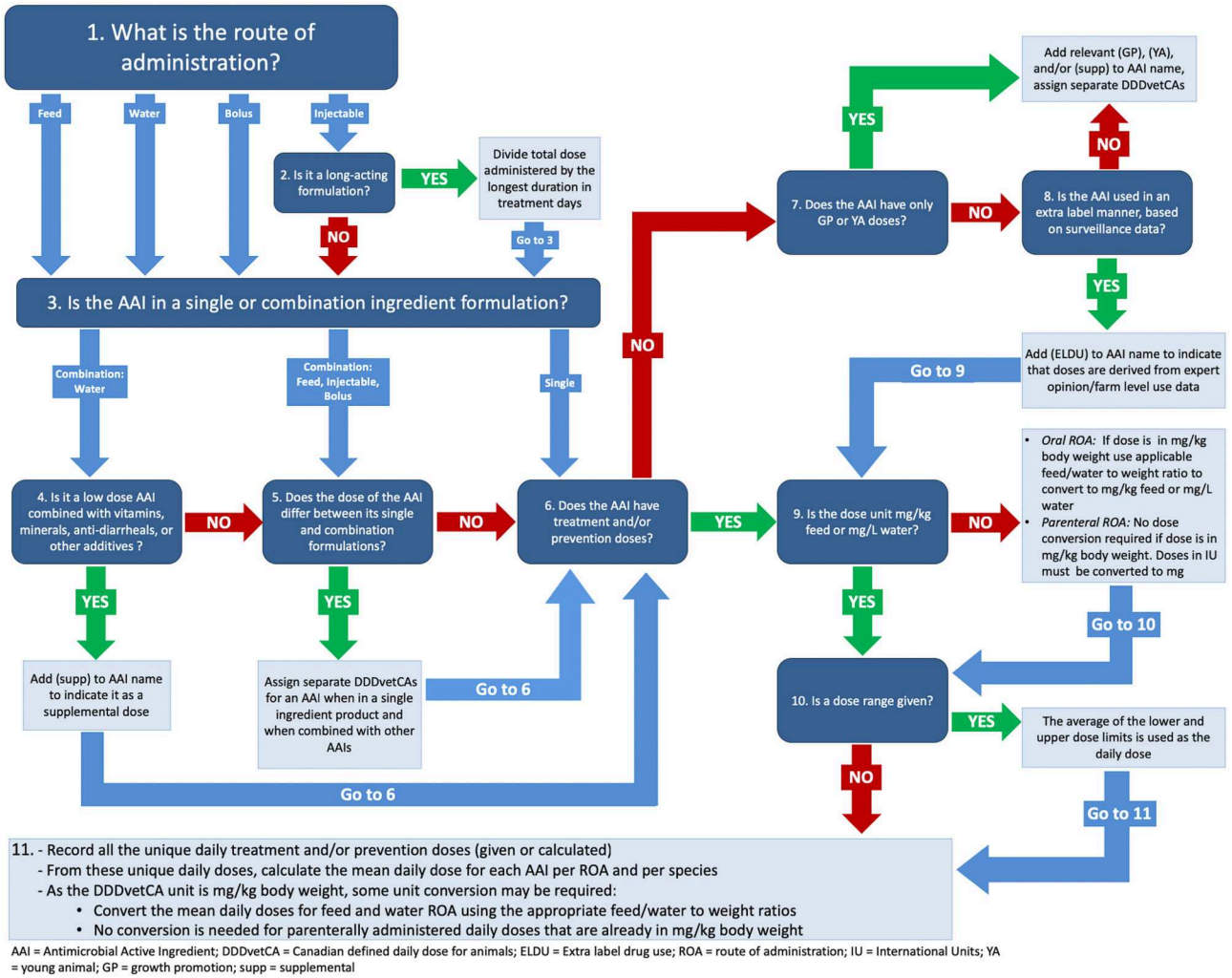

FIGURE 1 | A decision tree illustrating the process of assigning Canadian defined daily doses for animals (DDDvetCAs).

TABLE 1 | Example calculation of the mean daily dose and the Canadian defined daily dose for animals (DDDvetCA) for selected antimicrobial active ingredients and routes of administration, in poultry and pigs.

Species	Antimicrobial active ingredient	ROA[a]	Number of products marketed in Canada[b]	Unique dosages	Mean daily dose	Feed or water WCR[c,d]	DDDvetCA (mg/kg BW/day)[e]
Pigs	Chlortetracycline	Feed	12	55, 110, 220, 656	260.3 mg/kg feed	0.04	10.4
	Tylosin	Injectable	1	5.5	5.5 mg/kg bodyweight	N/A[f]	5.5
	Tylosin	Water	2	83, 250	166.5 mg/L water	0.1	16.7
Poultry	Bacitracin	Feed	3	55, 82.5, 110	77.9 mg/kg feed	0.13	10.1
	Amoxicillin	Water	2	52	52.0 mg/L water	0.23	12.0

[a] *Route of administration.*
[b] *Canadian Animal Health Institute (16).*
[c] *WCR, weight conversion ratio in kg feed/kg animal or L water/kg animal.*
[d] *European Medicines Agency (9).*
[e] *BW, body weight.*
[f] *Not applicable.*

antimicrobials occurs, category I antimicrobials could be substituted; there are no substitutes for antimicrobials in category I (19). Category III antimicrobials are less essential due to the availability of alternatives in categories I and II (19). Category IV antimicrobials include flavophospholipols and ionophores, which are not currently used in human medicine (19).

Assignment of DDDvetCAs

The DDDvetCAs, in mg/kg animal body weight per day, were assigned by calculating the mean of the tabulated unique daily doses for each AAI, stratified by species and route of administration. Examples of mean daily dose calculations can be found in **Table 1**. The mean daily dose was assigned as the DDDvetCA for that antimicrobial and route of administration. Generally, an AAI was assigned one DDDvet per route of administration, except for some AAIs used in combination. Following ESVAC's guidelines, when the dose of an AAI differed between single and combination ingredient use, due to a synergistic effect, the AAI was assigned two separate DDDvetCAs (9). One DDDvetCA was assigned based on the daily dose for single ingredient use and a second DDDvetCA was assigned based on the daily dose for combination ingredient use. For example, in poultry, the single ingredient formulation dose for lincomycin in water was 16 mg/L, while the lincomycin-spectinomycin formulation dose for lincomycin in water was 277.5 mg/L (16). In this case, two DDDvets were calculated, one for single use lincomycin, and one for lincomycin when administered as a combination product containing both lincomycin and spectinomycin. We followed ESVAC's convention of identifying DDDvets for antimicrobials used in combination as: *antimicrobial 1_antimicrobial 2*, which is understood as the DDDvetCA for antimicrobial 1 when it is used in combination with antimicrobial 2. In a similar situation, long-acting injectable ceftiofur in pigs was assigned a separate DDDvetCA from conventional injectable ceftiofur as the daily dose between the two formulations differed (1 and 3 mg/kg, respectively). Finally, consistent with EMA guidelines (9), we assigned prodrugs their own DDDvetCAs (e.g., procaine benzylpenicillin).

Specific decisions were required for doses for animals in specific age categories. Most antimicrobial products approved for use in feed and water did not differentiate doses by the age or production stage of the animal. However, some injectable and oral bolus product information included doses specific to young animals. For example, injectable gentamicin had doses for young pigs and chicks only (16). In addition, AMU data collected in Canada may be stratified by production stage in some species (e.g., farrowing, nursery or grow-finish stage in pigs). For this reason, we used these young animal doses to assign separate DDDvetCA specific to young animals, where applicable, which is a difference between our approach and that of EMA (9).

These young animal doses were often reported as a "per animal" dose. To obtain a dose in mg/kg these doses were divided by the average weight of the animal at treatment (16). For chicks and turkey poults, this weight was obtained from expert opinion (0.042 and 0.06 kg, respectively) (Agnes Agunos, personal communication, 2016). For piglets, we used ESVAC's standard piglet weight (4 kg) (9), which we confirmed to be consistent with Canadian pig production by expert opinion (Anne Deckert, personal communication, 2016). These young animal DDDvetCAs were labeled as such, to identify them as separate from the regular DDDvetCAs.

Another decision was required for handling products containing a mixture of various ingredients, such as anti-diarrheals, vitamins, minerals, and other additives, combined with very low doses of antimicrobials (16). The degree to which these products are used in pig production is not known, and with upcoming regulatory changes to require prescriptions for all medically important antimicrobials, these products may change or cease to be available (20). For these reasons, we assigned separate DDDvetCAs for the AAIs in these products. As with the young animal DDDvetCAs, the DDDvetCAs assigned using these low, or supplemental, doses were clearly labeled as such.

Comparison Between DDDvetCAs and EMA's DDDvets

Decisions made by EMA and CIPARS differed in how the DDDs for animals were stratified by route of administration, making comparisons between the two sets of DDDs challenging (9). While acknowledging that the differences in stratification by route of administration could contribute to differences between the DDDvetCAs and the DDDvets, we compared feed, water and bolus DDDvetCAs to oral DDDvets that matched by AAI. We also compared injectable DDDvetCAs with parenteral DDDvets that matched by AAI.

Comparisons between DDDvetCAs and DDDvets were made by calculating the ratio of the DDDvetCA to the DDDvet using Equation (2) for each matching DDDvet by species and route of administration. Ratios of one (±10%) were considered equal. Ratios above 1.1 indicated the DDDvetCA was larger than DDDvet, while ratios below 0.9 indicated the DDDvetCA was smaller. Ratios above 1.5 and below 0.5, indicating a more than 50% disparity between the two values, were considered to indicate a substantial difference between the two standards.

$$Ratio\ DDDvetCA : DDDvet = DDDvetCA / DDDvet \quad (2)$$

RESULTS

Poultry

An examination of the distribution of daily AAI doses showed that, for poultry, doses often varied widely for a given AAI (**Table 2**). An example of the variation in doses was sulfamethazine for administration through water, with a daily dose range of 143.8–335.4 mg/kg (**Table 2**). Sixty-seven percent of the DDDvetCAs included products with a single dose for all indications. Seventy-eight percent of the median and mean daily doses were equal; notable exceptions included amprolium in feed (mean dose = 20.7, median = 16.3, mg/kg/day) (**Table 2**). When the mean and median dose differed, the median dose was smaller than the mean dose.

Antimicrobial Products and AAIs

The distribution of antimicrobial products by route of administration is illustrated in **Figure 2**. We did not identify any antimicrobial products for use by individual oral treatment (bolus), as poultry are generally not given individual oral treatments. Most products and AAI were for use in-feed (**Figures 2**, **3**). Six in feed AAIs were ionophores and eight were

TABLE 2 | The minimum, maximum, and median doses for all antimicrobial active ingredients for which Canadian defined daily doses for animals (DDDvetCAs) were assigned for poultry, by route of administration, and the number of products used to assign each DDDvetCA.

Route of admin	Antimicrobial active ingredient[a,b]	Minimum dose	Maximum dose	Median dose	Ratio mean: median dose[c]	Number of products
Feed	Amprolium	13.3	32.5	16.3	1.27	1
Feed	Avilamycin	2.9	2.9	2.9	1.00	1
Feed	Bacitracin	7.2	13.1	10.1	1.00	3
Feed	Bambermycin (GP)	0.3	0.3	0.3	1.15	1
Feed	Chlortetracycline	7.2	28.6	14.3	1.17	3
Feed	Clopidol	16.3	16.3	16.3	1.00	1
Feed	Decoquinate	3.9	3.9	3.9	1.00	1
Feed	Diclazuril	0.1	0.1	0.1	1.00	1
Feed	Erythromycin	28.6	28.6	28.6	1.00	1
Feed	Halofuginone	0.4	0.4	0.4	1.00	1
Feed	Lasalocid	13.0	13.7	13.3	1.00	2
Feed	Maduramicin ammonium	0.7	0.7	0.7	1.00	1
Feed	Monensin	13.0	13.0	13.0	1.00	3
Feed	Narasin	9.1	9.1	9.1	1.00	2
Feed	Narasin_nicarbazin	5.2	5.2	5.2	1.00	1
Feed	Nicarbazin	10.4	26.0	16.3	1.06	1
Feed	Nicarbazin_narasin	5.2	5.2	5.2	1.00	1
Feed	Oxytetracycline	7.2	28.6	14.3	1.17	8
Feed	Benzylpenicillin (GP)	0.3	0.3	0.3	1.05	2
Feed	Procaine benzylpenicillin	5.4	5.4	5.4	1.00	2
Feed	Robenidine	4.3	4.3	4.3	1.00	1
Feed	Salinomycin	7.8	7.8	7.8	1.00	5
Feed	Semduramicin	3.3	3.3	3.3	1.00	1
Feed	Sulfadiazine_trimethoprim (ELDU)	10.8	10.8	10.8	1.00	1
Feed	Trimethoprim_sulfonamide (ELDU)	2.2	2.2	2.2	1.00	1
Feed	Tylosin	26.0	26.0	26.0	1.00	4
Feed	Virginiamycin	2.9	2.9	2.9	1.00	4
Feed	Zoalene (Dinitolmide)	16.3	24.3	20.3	1.00	1
Water	Amoxicillin	12.0	12.0	12.0	1.00	2
Water	Amprolium	55.2	55.2	55.2	1.00	1
Water	Apramycin (ELDU)	23.0	23.0	23.0	1.00	1
Water	Enrofloxacin (ELDU)	5.8	5.8	5.8	1.00	1
Water	Erythromycin	13.3	26.6	19.9	1.00	1
Water	Lincomycin	3.7	3.7	3.7	1.00	2
Water	Lincomycin_spectinomycin	63.8	63.8	63.8	1.00	2
Water	Neomycin	8.1	55.8	20.4	1.07	8
Water	Oxytetracycline	5.6	40.9	18.6	1.01	11
Water	Benzylpenicillin	41.0	41.0	41.0	1.00	4
Water	Benzylpenicillin (supp)	3.8	3.8	3.8	1.00	3
Water	Pyrimethamine_sulfaquinoxaline	3.4	3.4	3.4	1.00	1
Water	Spectinomycin_lincomycin	127.7	127.7	127.7	1.00	2
Water	Streptomycin (supp)	19.6	19.6	19.6	1.00	3
Water	Sulfamethazine	143.8	335.4	230.0	1.03	4
Water	Sulfaquinoxaline	58.4	87.5	72.9	1.00	2
Water	Sulfaquinoxaline_pyrimethamine	11.2	11.2	11.2	1.00	1
Water	Tetracycline	11.1	40.9	20.4	1.05	13
Water	Tylosin	28.8	115.0	71.9	1.00	2
Injectable	Ceftiofur (ELDU) (YA)	2.6	2.6	2.6	1.00	1

(Continued)

TABLE 2 | Continued

Route of admin	Antimicrobial active ingredient[a,b]	Minimum dose	Maximum dose	Median dose	Ratio mean: median dose[c]	Number of products
Injectable	Gentamicin (YA)	4.8	16.8	10.8	1.00	1
Injectable	Lincomycin_spectinomycin (ELDU) (YA)	6.0	6.0	6.0	1.00	1
Injectable	Spectinomycin_lincomycin (ELDU) (YA)	12.0	12.0	12.0	1.00	1

[a] *ELDU, based on known extra-label drug use doses in circumstances where an antimicrobial is not licensed for use in this species, yet surveillance data has documented the use of this antimicrobial; GP, based on growth promotion doses as no treatment/prevention doses exist; YA, based on doses indicated for young animals; supp, based on doses from multiple ingredient products with low doses of antimicrobials.*

[b] *Antimicrobial active ingredients written as: Active ingredient 1_active ingredient 2 = DDDvetCA for active ingredient 1 when used in combination with active ingredient 2.*

[c] *Ratio mean:median dose = DDDvetCA/median dose.*

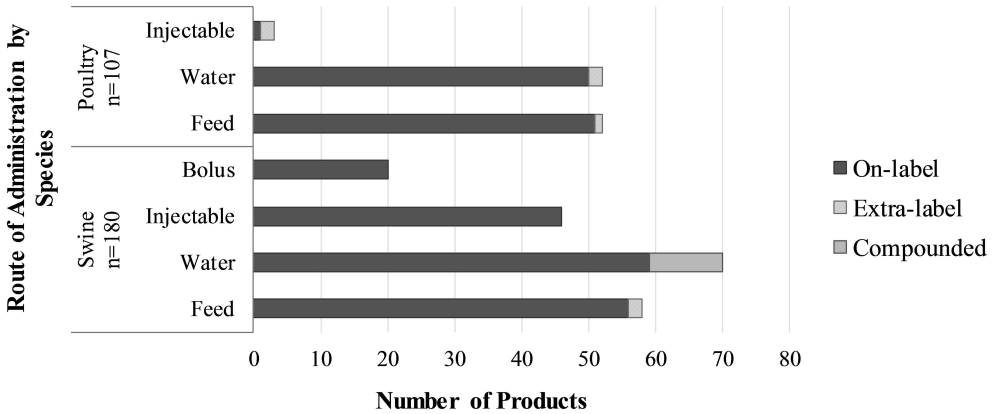

FIGURE 2 | The number of antimicrobial products used to assign Canadian defined daily doses for animals (DDDvetCAs) for pigs and poultry, stratified by route of administration (feed, water, injectable, bolus).

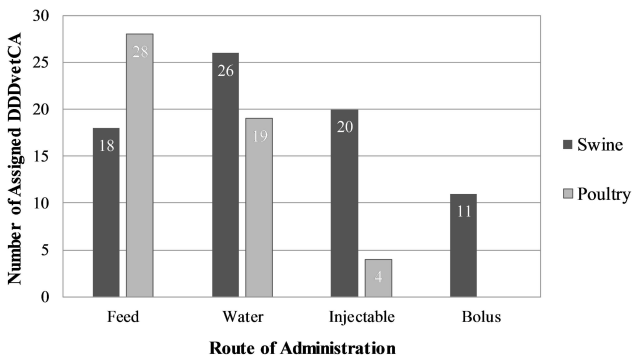

FIGURE 3 | The number of assigned Canadian defined daily doses for animals (DDDvetCAs) by species and route of administration, including coccidiostats and ionophores.

synthetic coccidiostats. The AAI with the most products was oxytetracycline (19 products).

DDDvetCAs

The complete list of DDDvetCAs assigned for poultry can be found in **Table 3**. Feed was the route of administration with the most assigned DDDvetCAs (**Figure 3**). Two of the in feed DDDvetCAs (bambermycin and benzylpenicillin) were based only on growth promotion doses (**Table 3**). All four of the injectable DDDvetCAs were assigned for young chicks/poults only (**Table 3**). Three of these young animal injectable DDDvetCAs (ceftiofur, lincomycin-spectinomycin, spectinomycin-lincomycin) were based on extra-label drug use (ELDU) doses as the injectable products containing these AAIs do not include doses for poultry in the product information, however, surveillance data indicate use in the hatcheries. Other DDDvetCAs assigned from extra-label use doses included enrofloxacin and apramycin in water, and trimethoprim-sulfadiazine in feed (**Table 3**). The DDDvetCA for injectable gentamicin was assigned based on subcutaneous doses for chicks and poults from the product information, although gentamicin may be used off-label *in-ovo*. No DDDvetCAs were assigned for poultry based on compounded product doses at this time (**Table 3**).

Comparison Between DDDvetCAs and EMA's DDDvets

In poultry, comparisons between DDDvetCAs and DDDvets were only possible for the feed and water routes of administration, as the European Union/European Economic Area countries do not have any parenteral antimicrobials products approved for poultry. Nineteen DDDvetCAs

TABLE 3 | The Canadian defined daily doses for animals (DDDvetCA) in $mg/kg_{poultry}$/day for antimicrobials used in poultry production, by antimicrobial active ingredient and route of administration.

Antimicrobial active ingredient[a,b]	DDDvetCA (mg/kg/day)
FEED	
Amprolium	20.7
Avilamycin	2.9
Bacitracin	10.1
Bambermycin (GP)	0.3
Chlortetracycline	16.7
Clopidol	16.3
Decoquinate	3.9
Diclazuril	0.1
Erythromycin	28.6
Halofuginone	0.4
Lasalocid	13.3
Maduramicin ammonium	0.7
Monensin	13
Narasin	9.1
Narasin_nicarbazin	5.2
Nicarbazin	17.2
Nicarbazin_narasin	5.2
Oxytetracycline	16.7
Penicillin G (GP)	0.3
Procaine penicillin G	5.4
Robenidine	4.3
Salinomycin	7.8
Semduramicin	3.3
Sulfadiazine_trimethoprim (ELDU)	10.8
Trimethoprim_sulfadiazine (ELDU)	2.2
Tylosin	26
Virginiamycin	2.9
Zoalene (Dinitolmide)	20.3
WATER	
Amoxicillin	12
Amprolium	55.2
Apramycin (ELDU)	23
Enrofloxacin (ELDU)	5.8
Erythromycin	19.9
Lincomycin	3.7
Lincomycin_spectinomycin	63.8
Neomycin	27.3
Oxytetracycline	18.8
Penicillin G	41
Penicillin G (supp)	3.8
Pyrimethamine_sulfaquinoxaline	3.4
Spectinomycin_lincomycin	127.7
Streptomycin (supp)	19.6
Sulfamethazine	236.4
Sulfaquinoxaline	72.9
Sulfaquinoxaline_pyrimethamine	11.2
Tetracycline	21.4
Tylosin	71.9
INJECTABLE	
Ceftiofur (ELDU) (YA)	2.6
Gentamicin (YA)	10.8
Lincomycin_spectinomycin (ELDU) (YA)	6
Spectinomycin_lincomycin (ELDU) (YA)	12

[a] DDDvetCA, Canadian defined daily dose for animals; LA, long-acting; YA, young animal doses; supp, supplements; ELDU, extra-label drug use; GP, growth promotion dose.
[b] Antimicrobial active ingredients written as: Active ingredient 1_active ingredient 2 = DDDvetCA for active ingredient 1 when used in combination with active ingredient 2.

could be compared to DDDvets (**Table 4**). For 34 feed and water DDDvetCAs there were no corresponding DDDvets for comparison.

Results of the comparison showed some similarities and differences between the two sets of DDD for animals. **Table 5** shows the frequency and proportion of DDDvetCA that were larger, smaller, or equivalent to their corresponding DDDvet according to the DDDvetCA/DDDvet ratio. More specifically, the DDDvetCAs and the DDDvets for erythromycin, neomycin and tylosin in water were similar with ratios between 0.9 and 1.1. Overall for poultry, five (26%) of the DDDvetCAs were larger and 11 (58%) of the DDDvetCAs were smaller than the corresponding DDDvets. DDDvetCAs that were notably different from the DDDvet included lincomycin combined with spectinomycin. Overall, nine DDDvetCAs (47%) differed by more than 50% from the equivalent DDDvets, with ratios <0.5 or >1.5.

Other Observations

An examination of the Health Canada categorization of all DDDvetCAs in poultry revealed two Health Canada Category I AAIs, namely ceftiofur and enrofloxacin, which are used extra-label in poultry. Thirteen AAIs were Category II, with the remainder in Categories III and IV, or uncategorized (19). Uncategorized AAIs included avilamycin (an orthosomycin antimicrobial), tiamulin (a pleuromutilin antimicrobial), pyrimethamine (an anti-protozoal usually combined with sulfaquinoxaline), and the chemical coccidiostats.

Pigs

As observed for poultry, an examination of the distribution of daily AAI doses showed that, for pigs, doses often varied widely for a given AAI. Like poultry, an example of a wide difference between the minimum and maximum daily dose of an AAI in pig was sulfamethazine in water, with minimum and maximum daily doses of 7 and 135 mg/kg, respectively (**Table 6**). Oxytetracycline, chlortetracycline, and bacitracin in feed had mean daily doses that varied from the median by a ratio of >1.5 (**Table 5**). Eighty-one percent of the median daily doses were identical to the median. Like poultry, the median dose was smaller than the mean dose in pigs, except for tiamulin in feed and neomycin (supplemental) bolus (**Table 6**).

TABLE 4 | The ratio of the Canadian defined daily doses for animals (DDDvetCA) by water, feed and oral bolus routes of administration to the European Medicine Agency's defined daily dose for animals (DDDvet) by the oral route of administration in poultry.

Antimicrobial active ingredient[a,b]	DDDvetCA	Canadian ROA	DDDvet[c]	EMA ROA[d]	Ratio[e]
Amoxicillin	12	Water	16	Oral	0.8
Apramycin	23	Water	81	Oral	**0.3**
Chlortetracycline	16.7	Feed	30	Oral	0.6
Enrofloxacin	5.8	Water	10	Oral	0.6
Erythromycin	28.6	Feed	20	Oral	1.4
Erythromycin	19.9	Water	20	Oral	1.0
Lincomycin	3.7	Water	8.6	Oral	**0.4**
Lincomycin_spectinomycin	63.8	Water	22	Oral	**2.9**
Neomycin	27.3	Water	24	Oral	1.1
Oxytetracycline	16.7	Feed	39	Oral	**0.4**
Oxytetracycline	18.8	Water	39	Oral	0.5
Spectinomycin_lincomycin	127.7	Water	38	Oral	**3.4**
Sulfadiazine_trimethoprim	10.8	Feed	34	Oral	**0.3**
Sulfamethazine	236.4	Water	182	Oral	1.3
Sulfaquinoxaline	72.9	Water	60	Oral	1.2
Tetracycline	21.4	Water	71	Oral	**0.3**
Trimethoprim_sulfadiazine	2.2	Feed	6.4	Oral	**0.3**
Tylosin	26	Feed	81	Oral	**0.3**
Tylosin	71.9	Water	81	Oral	0.9

Ratios above 1.5 and below 0.5, indicating substantial differences in these standardized doses, are in bold print.
[a] *Antimicrobial active ingredients written as: Active ingredient 1_active ingredient 2 = DDDvetCA for active ingredient 1 when used in combination with active ingredient 2.*
[b] *ELDU, extra-label drug use.*
[c] *European Medicines Agency (12).*
[d] *The EMA combined in feed, in water and oral bolus routes of administration into one oral DDDvet.*
[e] *Ratio, DDDvetCA/DDDvet.*

TABLE 5 | The frequency and proportion of Canadian defined daily doses for animals (DDDvetCA) that were larger, smaller, or equivalent to their corresponding defined daily dose for animals (DDDvet[a]), by species and route of administration.

Species	Route of administration	DDDvetCA:DDDvet Ratio >1.1 *N* (%)	DDDvetCA:DDDvet Ratio <0.9 *N* (%)	DDDvetCA:DDDvet Ratio ≥ 0.9 and ≤1.1 *N* (%)
Poultry	Feed	1 (17)	5 (83)	0 (0)
Poultry	Water	4 (31)	6 (46)	3 (23)
Pigs	Feed	0 (0)	11 (100)	0 (0)
Pigs	Water	5 (29)	7 (41)	5 (29)
Pigs	Injectable	5 (29)	6 (35)	6 (35)
Pigs	Bolus[b]	2 (33)	3 (50)	1 (17)
Poultry and pigs	All routes of administration	17 (24)	38 (54)	15 (21)

DDDvetCAs were considered larger when the ratio of the DDDvetCA/DDDvet was larger than 1.1, smaller when the ratio was <0.9, and equivalent when the ratio was equal to or between 0.9 and 1.1.
[a] *European Medicines Agency (12).*
[b] *Bolus, administered as individual oral treatment.*

Antimicrobial Products and AAIs

The distribution of antimicrobial products by route of administration is illustrated in **Figure 2**. Most products (including ELDU and compounded products) and AAIs were for use in water (**Figures 2**, **3**). Two in feed AAIs were ionophores and one was a synthetic coccidiostat. Like poultry, the AAI for which there were the most products was oxytetracycline (36 products).

DDDvetCAs

The complete list of assigned DDDvetCAs for pigs can be found in **Table 7**. The route of administration with the most assigned DDDvetCAs was in water (**Figure 3**). Two in water and three in feed DDDvetCAs were assigned based on growth promotion doses only, as these AAIs lacked doses for treatment or prevention (**Table 7**). Young animal DDDvetCAs were assigned for eleven bolus and four injectable AAIs (**Table 7**).

TABLE 6 | The minimum, maximum, and median doses for all antimicrobial active ingredients for which Canadian defined daily doses for animals (DDDvetCAs) were assigned for pigs, by route of administration, and the number of products used to assign each DDDvetCA.

Route of admin[a]	Antimicrobial active ingredient[b,c]	Minimum dose	Maximum dose	Median dose	Ratio mean: median dose[d]	Number of products
Feed	Avilamycin	3.2	3.2	3.2	1.00	1
Feed	Bacitracin	1.6	11.0	2.8	1.61	4
Feed	Bambermycin (ELDU) (GP)	0.1	0.1	0.1	1.00	1
Feed	Chlortetracycline	2.2	26.2	6.6	1.58	12
Feed	Lincomycin	1.8	8.8	4.4	1.14	6
Feed	Lincomycin_spectinomycin	0.9	0.9	0.9	1.00	2
Feed	Narasin (GP)	0.6	0.6	0.6	1.00	2
Feed	Oxytetracycline	2.0	22.0	4.4	1.73	13
Feed	Benzylpenicillin	0.6	2.2	1.1	1.18	6
Feed	Procaine benzylpenicillin (ELDU)	13.2	13.2	13.2	1.00	1
Feed	Salinomycin (GP)	1.0	1.0	1.0	1.00	2
Feed	Spectinomycin_lincomycin	0.9	0.9	0.9	1.00	2
Feed	Sulfamethazine	4.4	4.4	4.4	1.00	5
Feed	Tiamulin	1.5	8.8	6.3	0.90	4
Feed	Tilmicosin	8.0	16.0	12.0	1.00	2
Feed	Tylosin	1.8	4.4	3.1	1.00	7
Feed	Tylvalosin	1.7	1.7	1.7	1.00	1
Feed	Virginiamycin	2.2	4.4	3.3	1.00	4
Water	Amoxicillin	16.0	16.0	16.0	1.00	2
Water	Ampicillin (C)	20.0	20.0	20.0	1.00	1
Water	Apramycin	10.0	10.0	10.0	1.00	1
Water	Gentamicin (C)	1.1	1.1	1.1	1.00	1
Water	Lincomycin	3.3	3.3	3.3	1.00	2
Water	Lincomycin_spectinomycin	2.2	2.2	2.2	1.00	2
Water	Neomycin	7.0	17.8	12.5	1.00	9
Water	Oxytetracycline	5.0	33.3	13.6	1.07	11
Water	Benzylpenicillin	17.8	17.8	17.8	1.00	3
Water	Benzylpenicillin (supp) (GP)	3.6	3.6	3.6	1.00	3
Water	Phenoxymethylpenicillin (C)	18.4	38.0	28.2	1.00	3
Water	Spectinomycin_lincomycin	4.5	4.5	4.5	1.00	2
Water	Streptomycin (supp) (GP)	18.4	18.6	18.5	1.00	3
Water	Sulfadiazine_trimethoprim (C)	20.0	44.4	30.0	1.08	5
Water	Sulfamerazine (supp)	2.5	4.1	3.3	1.00	4
Water	Sulfamethazine	7.0	135.0	79.2	1.00	10
Water	Sulfamethazine (supp)	6.3	6.3	6.3	1.00	3
Water	Sulfapyridine	33.3	33.3	33.3	1.00	1
Water	Sulfathiazole	37.8	75.0	39.3	1.18	6
Water	Sulfathiazole (supp)	5.0	15.6	10.3	1.00	4
Water	Tetracycline	2.0	17.8	8.3	1.04	12
Water	Tiamulin	4.9	4.9	4.9	1.00	2
Water	Tilmicosin (C)	10.0	10.0	10.0	1.00	1
Water	Trimethoprim_sulfadiazine (C)	5.5	8.9	7.0	1.01	5
Water	Tylosin	8.3	25.0	16.7	1.00	2
Water	Tylvalosin	5.0	5.0	5.0	1.00	1
Injectable	Ampicillin	6.0	6.0	6.0	1.00	1
Injectable	Benzathine benzylpenicillin combi (LA)	1.2	1.2	1.2	1.00	1
Injectable	Ceftiofur	3.0	3.0	3.0	1.00	6
Injectable	Ceftiofur (LA)	1.0	1.0	1.0	1.00	1
Injectable	Enrofloxacin	7.5	7.5	7.5	1.00	1

(Continued)

TABLE 6 | Continued

Route of admin[a]	Antimicrobial active ingredient[b,c]	Minimum dose	Maximum dose	Median dose	Ratio mean: median dose[d]	Number of products
Injectable	Florifenicol	7.5	7.5	7.5	1.00	1
Injectable	Gentamicin (YA)	1.3	1.3	1.3	1.00	1
Injectable	Lincomycin	10.0	10.0	10.0	1.00	2
Injectable	Oxytetracycline	5.0	6.7	5.9	1.00	13
Injectable	Oxytetracycline (YA)	12.5	16.7	14.6	1.00	3
Injectable	Procaine benzylpenicillin	12.0	15.0	13.5	1.00	7
Injectable	Procaine benzylpenicillin (LA)	6.7	6.7	6.7	1.00	2
Injectable	Procaine benzylpenicillin_combi (LA)	1.5	1.5	1.5	1.00	1
Injectable	Sulfadoxine_trimethoprim	13.3	13.3	13.3	1.00	5
Injectable	Sulfadoxine_trimethoprim (YA)	25.0	25.0	25.0	1.00	5
Injectable	Tiamulin	11.0	11.0	11.0	1.00	1
Injectable	Trimethoprim_sulfadoxine	2.4	2.4	2.4	1.00	5
Injectable	Trimethoprim_sulfadoxine (YA)	5.0	5.0	5.0	1.00	6
Injectable	Tulathromycin (LA)	0.3	0.3	0.3	1.00	2
Injectable	Tylosin	5.5	5.5	5.5	1.00	1
Bolus	Neomycin (supp) (YA)	5	12.5	10	0.92	2
Bolus	Neomycin (YA)	8.9	33.3	17.8	1.11	6
Bolus	Oxytetracycline (YA)	5	55	18.9	1.39	9
Bolus	Spectinomycin (YA)	12.5	25.0	18.8	1.00	2
Bolus	Succinylsulfathiazole (supp) (YA)	24.0	48.0	36.0	1.00	1
Bolus	Sulfaguanidine (YA)	83.8	83.8	83.8	1.00	2
Bolus	Sulfamethazine (YA)	48.8	187.5	118.1	1.00	2
Bolus	Sulfanilamide (YA)	73.1	73.1	73.1	1.00	1
Bolus	Sulfathiazole (YA)	41.8	73.1	57.4	1.00	3
Bolus	Tetracycline (YA)	12.8	17.8	15.3	1.00	2
Bolus	Toltrazuril (YA)	20.0	20.0	20.0	1.00	1

[a] *Bolus, administered as individual oral treatment.*

[b] *ELDU, based on known extra-label drug use doses; GP, based on growth promotion doses as no treatment/prevention doses exist; C, based on compounded drug doses; LA, long acting; YA, based on doses indicated for young animals; supp, based on doses from multiple ingredient products with low doses of antimicrobials.*

[c] *Antimicrobial active ingredients written as: Active ingredient 1_active ingredient 2 = DDDvetCA for active ingredient 1 when used in combination with active ingredient 2. Exception: Benzathine Benzylpenicillin combi = Benzathine Benzylpenicillin in combination with any other antimicrobial active ingredient.*

[d] *Ratio mean dose:median dose = DDDvetCA/median dose.*

DDDvetCAs were assigned from ELDU doses for bambermycin and procaine benzylpenicillin administered through feed, as the in-feed products containing these AAIs do not include doses for pigs in their product information, however, surveillance indicates use by this route of administration. Unlike poultry, some DDDvetCAs were assigned using compounded product doses (**Table 7**).

Comparison Between DDDvetCAs and EMA's DDDvets

In pigs, comparisons between DDDvetCAs and DDDvets was possible for all routes of administration. Fifty-one DDDvetCAs could be compared to DDDvets (**Table 8**). The remaining 24 DDDvetCAs did not have any corresponding DDDvet.

As with poultry, results of the comparison showed some similarities and differences between the two sets of DDD for animals. **Table 5** shows the frequency and proportion of DDDvetCA that were larger, smaller, or equivalent to their corresponding DDDvet using the DDDvetCA/DDDvet ratio. More specifically, DDDvetCAs and DDDvets were similar (±10%) for water administered amoxicillin, lincomycin-spectinomycin, apramycin, and sulfamethazine, and for injectable ceftiofur, lincomycin, procaine benzylpenicillin, sulfadoxine_trimethoprim, and tiamulin. All feed DDDvetCAs were smaller than their corresponding DDDvet (**Table 5**). Overall for pigs, a difference of more than 50% was observed between 35% of the DDDvetCAs and their corresponding DDDvets (e.g., enrofloxacin injectable DDDvetCA = 7.5 mg/kg/day; DDDvet = 3.4 mg/kg/day).

Other Observations in Pigs

An examination of the Health Canada categorization for all DDDvetCAs in pigs revealed that two Health Canada Category I AAIs, namely ceftiofur and enrofloxacin, were licensed for use in pigs (19). Sixteen AAIs were Category II, with the remainder in Categories III and IV, or uncategorized (as for poultry).

TABLE 7 | The Canadian defined daily doses for animals (DDDvetCA) in mg/kg_{pig}/day for antimicrobials used in pig production, by antimicrobial active ingredient and route of administration.

Antimicrobial active ingredient[a,b]	DDDvetCA (mg/kg/day)
FEED	
Avilamycin	3.2
Bacitracin	4.5
Bambermycin (ELDU) (GP)	0.1
Chlortetracycline	10.4
Lincomycin	5.0
Lincomycin_spectinomycin	0.9
Narasin (GP)	0.6
Oxytetracycline	7.6
Penicillin G	1.3
Procaine Penicillin G (ELDU)	13.2
Salinomycin (GP)	1.0
Spectinomycin_lincomycin	0.9
Sulfamethazine	4.4
Tiamulin	5.7
Tilmicosin	12.0
Tylosin	3.1
Tylvalosin	1.7
Virginiamycin	3.3
INJECTABLE	
Ampicillin	6.0
Benzathine penicillin G-combi[c] (LA)	1.2
Ceftiofur	3.0
Ceftiofur (LA)	1.0
Enrofloxacin	7.5
Florifenicol	7.5
Gentamicin (YA)	1.3
Lincomycin	10.0
Oxytetracycline	5.9
Oxytetracycline (YA)	14.6
Procaine Penicillin G	13.5
Procaine Penicillin G (LA)	6.7
Procaine Penicillin G_combi[c] (LA)	1.5
Sulfadoxine_trimethoprim	13.3
Sulfadoxine_trimethoprim (YA)	25.0
Tiamulin	11.0
Trimethoprim_sulfadoxine	2.4
Trimethoprim_sulfadoxine (YA)	5.0
Tulathromycin (LA)	0.3
Tylosin	5.5
WATER	
Amoxicillin	16.0
Ampicillin (C)	20.0
Apramycin	10.0
Gentamicin (C)	1.1
Lincomycin	3.3
Lincomycin_spectinomycin	2.2
Neomycin	12.5
Oxytetracycline	14.6
Penicillin G	17.8
Penicillin G (supp) (GP)	3.6
Penicillin V (C)	28.2
Spectinomycin_lincomycin	4.5
Streptomycin (supp) (GP)	18.5
Sulfadiazine_trimethoprim (C)	32.4
Sulfamerazine (supp)	3.3
Sulfamethazine	79.0
Sulfamethazine (supp)	6.3
Sulfapyridine	33.3
Sulfathiazole	46.2
Sulfathiazole (supp)	10.3
Tetracycline	8.6
Tiamulin	4.9
Tilmicosin (C)	10.0
Trimethoprim_sulfadiazine (C)	7.1
Tylosin	16.7
Tylvalosin	5.0
BOLUS[d]	
Neomycin (supp) (YA)	9.2
Neomycin (YA)	19.7
Oxytetracycline (YA)	26.2
Spectinomycin (YA)	18.8
Succinylsulfathiazole (supp) (YA)	36.0
Sulfaguanidine (YA)	83.8
Sulfamethazine (YA)	118.1
Sulfanilamide (YA)	73.1
Sulfathiazole (YA)	57.4
Tetracycline (YA)	15.3
Toltrazuril (YA)	20.0

[a] DDDvetCA, Canadian defined daily dose for animals; LA, long-acting; YA, young animal; supp, supplements; ELDU, extra-label drug use; GP, growth promotion; C, compounded drug use.

[b] Antimicrobial active ingredients written as: Active ingredient 1_active ingredient 2 = DDDvetCA for active ingredient 1 when used in combination with active ingredient 2.

[c] Benzathine Penicillin G-combi and Procaine Penicillin G-combi (LA): when combined with any other antimicrobial active ingredient.

[d] Administered as individual oral treatments.

Overall Results

Across both species, more DDDvetCAs were assigned for AAIs used in pigs than for poultry (**Table 3**). There were 53 feed, water and bolus DDDvetCAs that could be matched by AAI to 40 oral DDDvets, and 17 injectable DDDvetCAs that could be matched by AAI to 14 parenteral DDDvets (**Tables 5**, **8**).

DISCUSSION

Assigning DDDvetCAs

Assigning DDDvetCAs was a resource intensive and iterative process, and regular group discussions were needed to make a range of operational decisions. Examples of these decisions include, among others, the setup of the spreadsheet used to

collect product information, how to interpret and handle various product information situations (such as combination products or dose ranges), and which doses to use for the determination of the mean dose (e.g., all doses or only unique doses). These decisions were sometimes revisited with the acquisition of new information.

Part of what made the DDDvetCA assignment resource intensive was the need for human resources to extract dose information from product information in the CVP and CMIB. At times, tracking down manufacturer and/or expert opinion was necessary where no licensed product dose information was available. Between 10 and 15 min were required to extract the required information from each product, provided the product information was comprehensive and clear. However, differences in the way product information and drug doses were written caused significant variation in the time needed to extract the information required. Some product information was clear and easy to understand, while others were more complex. Some products had doses for multiple indications, multiple species, or multiple routes of administration. For example, some water products included doses for individual animal dosing and for herd/flock dosing. The process became faster as familiarity with product information increased.

The decision to exclude growth promotion doses from the assignment of DDDvetCAs was consistent with EMA's guidelines, as in the European Union, the use of antimicrobial products for growth promotion is not permitted, and as a result, EMA's DDDvets are based on treatment and prevention doses only (9). In Canada, as of December 1, 2018, all antimicrobial products considered medically important by Health Canada will no longer be labeled for growth promotion purposes (20). By excluding growth promotion doses, the DDDvetCAs will remain relevant after this change. The decision to assign separate DDDvetCAs to growth promotion AAIs was based on the need to quantify their use, as these AAIs appear in Canadian surveillance data.

A departure from EMA's guidelines was the assignment of DDDvetCAs to AAIs used in an extra-label manner in the species of interest. Prescribing antimicrobials in an extra-label manner is legal for veterinarians in Canada, when no approved product for the species of interest exists (21). For the same reason that DDDvetCAs were needed for growth promotion AAIs, DDDvetCAs were needed for ELDU AAIs where surveillance data indicated their use in Canada. Since these extra-label DDDvetCAs are based on used doses, rather than labeled doses, they more closely resemble used daily doses (22). We recognize that assigning DDDvetCAs to these extra-label AAIs was a departure from defined daily dose methodology, however, due to the need to quantify the use of these AAIs we decided to include them in the DDDvetCA assignment.

We followed EMA's DDDvet guidelines for assigning separate DDDvetCAs to AAIs used in combination formulations, when their mean daily doses differed from single ingredient formulations (9). In contrast, the World Health Organization's methodology for the assignment of human DDDs assigns a single DDD to AAIs used in combination, using the mean daily dose of the main AAI ingredient only (23). When combination AAI products are used, the World Health Organization's method will only account for the use of the main ingredient, while CIPARS' (and EMA's) method will account for the use of each of the AAI in the combination product. This will ensure that all AAI use is considered for future modeling with AMR data.

The decision to use an average, or mean, daily dose to assign DDDvetCAs was also consistent with EMA's guidelines and with the DDD in human medicine (9, 10). While examining the distribution of AAI doses, we investigated using the median daily dose to assign DDDvetCAs. Over 80% of mean and median daily doses were identical in each species, so whether the mean or median dose was used made little difference to the resulting DDDvetCAs. Where differences existed, the mean was almost always larger than the median, which suggested that for these cases there may be some high dose outliers influencing the mean. Using the mean daily dose kept the DDDvetCAs more closely aligned with the EMA's methodology and the definition of a DDDvet (9). Examining AMU farm surveillance data to see if these outlying doses are in use may prove interesting.

Differences Between DDDvetCAs and EMA's DDDvets

A major difference between DDDvetCAs and DDDvets is the stratification by routes of administration for products administered orally. EMA grouped the oral routes of administration together when assigning DDDvets, creating one category called oral (9, 12), while at CIPARS, we assigned DDDvetCAs to each oral route of administration separately. This difference in stratification very likely contributed to the differences between the feed, water and bolus DDDvetCAs and the oral DDDvets. Assigning DDDvetCAs separately to each oral route of administration will enable CIPARS to monitor changes in use between these routes of administration. An argument could be made that the DDDvetCAs should not be compared to DDDvets, due to the differences in route of administration stratification. However, we felt that these comparisons would be made by others, and by including the comparison in this study we could emphasize the strengths and limitations of doing so. The differences found between our feed, water and bolus DDDvetCAs and the oral DDDvets may have been less evident if we combined the oral routes of administration together in a similar manner to EMA. Even with the differences in stratification, some of the feed, water and bolus DDDvetCAs were identical or very close to the corresponding oral DDDvets.

CIPARS' method of assigning DDDvetCAs by using only unique AAI doses to calculate the mean daily dose also differed from EMA's guidelines (9). EMA's method of using the minimum and maximum daily doses to determine the mean daily dose meant that the doses on either end of the dose range had a greater effect on the mean. In contrast, by using the range of unique doses, any doses in the middle of the range have a moderating effect on the mean dose. For example, the unique daily doses for chlortetracycline in feed are 55, 110, 220, and 656 mg per kg of feed. If we used EMA's method, the mean daily dose would be

TABLE 8 | The ratio of the Canadian defined daily doses for animals (DDDvetCA) by route of administration to the European Medicine Agency's defined daily dose for animals (DDDvet) in pigs.

Antimicrobial active ingredient[a]	DDDvetCA	Canadian ROA	DDDvet[b]	EMA ROA[c]	Ratio[d]
Amoxicillin	16.0	Water	17.0	Oral	0.9
Ampicillin	20.0[e]	Water	30.0	Oral	0.7
Ampicillin	6.0	Injectable	12.0	Parenteral	0.5
Apramycin	10.0	Water	9.0	Oral	1.1
Benzathine Penicillin G_combi (LA)	1.2	Injectable	5.4	Parenteral	**0.2**
Ceftiofur (LA)	1.0	Injectable	0.8	Parenteral	1.3
Ceftiofur	3.0	Injectable	3.0	Parenteral	1.0
Chlortetracycline	10.4	Feed	31.0	Oral	**0.3**
Enrofloxacin	7.5	Injectable	3.4	Parenteral	**2.2**
Florifenicol	7.5	Injectable	9.5	Parenteral	0.8
Gentamicin	1.1[e]	Water	1.4	Oral	0.8
Gentamicin	1.3[f]	Injectable	1.4	Parenteral	0.9
Lincomycin	5.0	Feed	7.6	Oral	0.7
Lincomycin	10.0	Injectable	10.0	Parenteral	1.0
Lincomycin	3.3	Water	7.6	Oral	**0.4**
Lincomycin_spectinomycin	0.9	Feed	2.2	Oral	**0.4**
Lincomycin_spectinomycin	2.2	Water	2.2	Oral	1.0
Neomycin	19.7[f]	Bolus	25.0	Oral	0.8
Neomycin	12.5	Water	25.0	Oral	0.5
Oxytetracycline	26.2[f]	Bolus	26.0	Oral	1.0
Oxytetracycline	14.6[f]	Injectable	7.5	Parenteral	**1.9**
Oxytetracycline	7.6	Feed	26.0	Oral	**0.3**
Oxytetracycline	5.9	Injectable	7.5	Parenteral	0.8
Oxytetracycline	14.6	Water	26.0	Oral	0.6
Penicillin G	1.3	Feed	48.0	Oral	**<0.1**
Penicillin G	17.8	Water	48.0	Oral	**0.4**
Procaine Penicillin G	13.5	Injectable	13.0	Parenteral	1.0
Spectinomycin	18.8[f]	Bolus	33.0	Oral	0.6
Spectinomycin_lincomycin	0.9	Feed	3.4	Oral	**0.3**
Spectinomycin_lincomycin	4.5	Water	3.4	Oral	1.3
Sulfadiazine_trimethorprim	32.4[e]	Water	23.0	Oral	1.4
Sulfadoxine_trimethoprim	13.6[f]	Injectable	14.0	Oral	1.0
Sulfaguanidine	83.8[f]	Bolus	54.0	Oral	**1.6**
Sulfamethazine	118.1[f]	Bolus	92.0	Oral	1.3
Sulfamethazine	4.4	Feed	92.0	Oral	**<0.1**
Sulfamethazine	79.0	Water	92.0	Oral	0.9
Tetracycline	15.3[f]	Bolus	49.0	Oral	**0.3**
Tetracycline	8.6	Water	49.0	Oral	**0.2**
Tiamulin	5.7	Feed	9.7	Oral	0.6
Tiamulin	11.0	Injectable	12.0	Parenteral	0.9
Tiamulin	4.9	Water	9.7	Oral	0.5
Tilmicosin	10.0[e]	Water	15.0	Oral	0.7
Tilmicosin	12.0	Feed	15.0	Oral	0.8
Trimethoprim_sulfadiazine	7.1	Water	4.7	Oral	1.5
Trimethoprim_sulfadoxine	5.0[f]	Injectable	4.7	Parenteral	1.1
Trimethoprim_sulfadoxine	2.4	Injectable	3.0	Parenteral	0.8
Tylosin	3.1	Feed	12.0	Oral	**0.3**
Tylosin	5.5	Injectable	13.0	Parenteral	**0.4**
Tylosin	16.7	Water	12.0	Oral	1.4

(Continued)

TABLE 8 | Continued

Antimicrobial active ingredient[a]	DDDvetCA	Canadian ROA	DDDvet[b]	EMA ROA[c]	Ratio[d]
Tylvalosin	1.7	Feed	3.6	Oral	0.5
Tylvalosin	5.0	Water	3.6	Oral	1.4

Ratios above 1.5 and below 0.5, indicating substantial differences in these standardized doses, are in bold print.
[a] *Antimicrobial active ingredients written as: Active ingredient 1_active ingredient 2 = DDDvetCA for active ingredient 1 when used in combination with active ingredient 2.*
[b] *European Medicines Agency. Defined daily doses for animals (DDDvet) and defined course doses for animals (DCDvet). 2016.*
[c] *The EMA combined in-feed, in-water and oral bolus routes of administration into one oral DDDvet.*
[d] *Ratio = DDDvetCA/DDDvet.*
[e] *DDDvetCA assigned using compounded doses.*
[f] *DDDvetCA assigned using young animal doses.*

355.5 mg per kg of feed and a DDDvet of 14.2 mg/kg/day, while using our method results in a mean daily dose of 260.3 mg/kg feed and a DDDvetCA of 10.4 mg/kg/day.

An example of yet another way of calculating the mean daily dose is Postma et al.'s (13) method of averaging every dose found, regardless of how often each dose appears in product information. Postma et al. (13) felt this method was the clearest but acknowledged that the number of products that contained a specific AAI influenced the mean. Since the number of products containing an AAI does not necessarily reflect the frequency of use, we opted for a more neutral approach and attributed equal weight to every unique dose reported in the CVP and CMIB (16, 17).

Another difference between CIPARS', EMA's, and Postma et al.'s (13) methodology is in the approach to young animal doses. EMA included both young and adult doses in the calculation of a single average daily dose that could then be applied to all ages of animals (9). Postma et al. (13) followed human medicine methodology by incorporating only adult doses in the assignment of their DDDvets. CIPARS chose to separate young animal doses from the rest and assign age stratified DDDvetCAs in those AAIs with young animal doses. This decision was made because age-stratified AMU data were available to CIPARS, or would be available in the future, making age-stratified DDDvetCAs useful.

Defined daily dose methodology in human medicine deals with differences in dosing by age by incorporating the weight of a standard adult (70 kg) into the assignment of the DDD (10). As a result, human DDD are assigned in mg/day, rather than mg/kg/day as in veterinary medicine (10). Consequently, the World Health Organization's guidelines for ATC classification and DDD assignment in humans states that DDDs in children ages >1 month to 18 years are impossible to assign, as pediatric doses are dependent on age and weight, which vary widely (10). An advantage of assigning DDD for animals in mg/kg/day rather than mg/day is that they can be applied to animals in various weight and/or age categories. Assigning specific young animal DDDvetCAs, where young animal doses exist, can help us avoid the challenges experienced in human medicine when measuring AMU in pediatrics (24).

There are many other reasons for the observed differences in DDD for animals between CIPARS and EMA, one of which is that EMA may have had a wider range of AAI doses to work with, due to the collection of AAI doses from nine European countries (9). However, fully elucidating all the reasons for the differences between the EMA's DDDvets and the DDDvetCAs was outside the scope of this project. We can speculate that different labeling regulations, different treatment indications, and different husbandry practices may all contribute. Ultimately, whether the DDDvet or the DDDvetCA for an AAI is higher or lower does not necessarily reflect the use of that AAI in practice. DDD for animals are intended to be a technical measurement only (9). They are useful when standardized doses are needed for monitoring of trends in AMU and other purposes, in a variety of populations, whether they be national or regional.

The Need for DDDvetCAs

The findings from this project confirmed the need for national DDDvetCAs for Canada for a few reasons. One reason was the observation that the DDDvets did not cover all the AAIs used in veterinary medicine in Canada. Also, while drawing conclusions from differences between DDDvetCAs and DDDvets assigned to oral routes of administration is difficult due to issues previously discussed, the differences observed between injectable DDDvetCAs and parenteral DDDvets appear to confirm the need for DDDvetCA that reflect antimicrobial selection pressure in a Canadian context.

The assigned DDDvetCAs have already been used by CIPARS for reporting farm-level surveillance data (5, 25). In the annual CIPARS report, the DDDvetCAs were used in the calculation of dose-based AMU indicators such as the number of defined daily doses for animals per 1,000 animal-days (26), and the number of defined daily doses for animals per population correction unit (5). Indicators such as these that use the DDDvetCAs will be valuable for in-country application to Canadian AMU data.

However, when comparing AMU between countries, using country specific DDD for animals such as the DDDvetCAs, may not be appropriate, due to the same challenges we observed when comparing DDDvetCAs and DDDvets. Differences in methodology and in antimicrobials authorized for use, among other issues, means that when reporting AMU internationally, it would be preferable for all reporting countries to use a set of international DDD for animals assigned using a single methodology. Ideally, these international DDD for animals would be assigned from globally represented product doses. Hence, the objectives of the reporting, whether national or international, will determine the choice of whether to use country-specific or international DDD for animals.

Limitations

A limitation of the DDDvet methodology is that they are based on AAI doses from product information, which may not reflect the use of the AAI in practice (9, 10). When measuring AMU from surveillance data, where dosing practices may vary widely, the assigned DDDvets provide a consistent and transparent technical method for adjusting weight-based measures of AMU by dose. Where more specific information on AMU exposure is required, using used daily doses (UDD) may be more appropriate, noting that the results obtained from such an analysis will specific to the population from which the UDD were determined (22). Using UDD would require detailed data, including used doses and animal weights at treatment, and the results would be specific to a population at a point in time, as used doses and dosing practices frequently change.

The DDDvetCAs will need to be reviewed periodically as product doses may change, new products may be registered, or older ones discontinued. Also, new indications for use may be added to product information and changes in approved species may occur. While a DDDvetCA may be subject to review in specific instances, the aim is for the assigned DDDvetCAs to remain stable over time. This stability over time will allow for AMU trends to be followed long-term without frequent changes that will complicate analyses and interpretation. Future revisions will be aided by the Microsoft Excel® 2010 spreadsheet designed and used for tabulation and calculation of the DDDvetCAs, which will function as a database. To make future revisions easier, the development of an automated product registration system to flag product dose changes or new products would be helpful.

CONCLUSION

The study of AMU is essential, enabling the examination of the impact on animal and human health due to the extent, nature, and determinants of AMU, and due to the associations between AMU and AMR. The DDDvetCAs will be valuable in the study of AMU in Canada, and while the process of assigning DDDvetCAs for the first time was challenging and resource intensive, maintaining them will require fewer resources. EMA's published principles for assigning DDDvets were an invaluable source of guidance and information for the creation of DDDvetCAs (9). Future steps for CIPARS include exploring DDDvetCA assignment for other production types such as cattle (beef and dairy), veal, and farmed fish.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Antimicrobial Drug Consumption on Swiss Pig Farms: A Comparison of Swiss and European Defined Daily and Course Doses in the Field

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Defined Daily Doses (DDD) and Defined Course Doses (DCD) have been established in both human and veterinary medicine in order to standardize the measurement of treatments in a population. In 2016 the European Medicines Agency published average defined daily dose (DDDvet) and defined course dose (DCDvet) values for antimicrobial agents used in livestock production. Similarly, national defined doses (DDDch and DCDch) for the pig sector in Switzerland have recently been determined. The aim of this study was to compare the outcome of calculating antimicrobial consumption based on either DDDvet/DCDvet or DDDch/DCDch. Data from 227 Swiss pig farms describing antimicrobial use in 2015 was collected. The numbers of treatment days and treatments were calculated using DDDvet/DCDvet and DDDch/DCDch respectively, for each farm in total and for different antimicrobial classes. Associations between calculated numbers of DDDvet/DCDvet and DDDch/DCDch on farm level were investigated. In addition, differences concerning antimicrobial use were investigated between different production types of farms (piglet-producer, finishing farm or farrow-to-finishing farm). Using DDDch/DCDch values we calculated 1,805,494 treatment days and 433,678 treatments compared to 1,456,771 treatment days (19% ratio) and 303,913 treatments (30% ratio) based on DDDvet/DCDvet. Penicillins (21.4/26.6%), polypeptides (18.6/27.6%) and fluoroquinolones (9.5/8.8%) were the most frequently used classes of antimicrobials based on calculation using both DDDch and DDDvet. Similar findings were observed for complete treatments (DCDch/vet) (penicillins: 52.8/39.6%; polypeptides: 7.8/14.2%; fluoroquinolones: 13.2/12.9%). The number of treatment days or treatments per farm was higher for piglet-producers and farrow-to-finishing farms compared to finisher farms regardless of whether Swiss or European DDD or DCD values were used for the calculation (each $P < 0.001$). Similar results for antimicrobial use (AMU) obtained at farm level were observed when calculated either by Swiss or European definitions. Nevertheless, marked differences could be observed in the assessment of the use of specific antimicrobial classes in the field based on DDDvet/DCDvet compared to DDDch/DCDch.

Keywords: antimicrobial drug usage, antimicrobial classes, defined daily dose, defined course dose, European medicines agency, monitoring systems, pigs, Switzerland

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INTRODUCTION

Antimicrobial use (AMU) is associated with the selection of resistant pathogens (1, 2) and the spread of resistance both within and between human and veterinary medicine (3–5). Responsible use of antimicrobials is therefore essential (6).

The importance of antimicrobial resistance for public health is internationally acknowledged (7, 8) and AMU in food-producing animals is monitored by various authorities (9, 10) in order to determine trends in resistance development.

In addition to monitoring systems measuring the amount of active ingredients, systems based on application equivalents have been established in several countries to monitor AMU in veterinary medicine (11–13). These application equivalents, originally developed for humans (14), standardize the measurement of AMU (15), by taking into account the dosages of the various antimicrobial compounds, and defining a dosage required daily or for a whole treatment. In line with the formal definition of the World Health Organization (WHO), Defined Daily Doses (DDD) and Defined Course Doses (DCD) are the assumed average maintenance doses per day or total treatment duration (16), which allow the estimation of number of treatment days respectively, number of treatments in a population (17).

In 2016, following these principles, the European Medicines Agency (EMA) published average defined daily dose (DDDvet) and defined course dose (DCDvet) values for antimicrobial agents used in livestock production as a tool to facilitate standardized collection and presentation of AMU among European member states (18). These values were defined by calculating the mean of given registrations for livestock production from nine different European countries. In analogy with the principles of the EMA (19), national defined doses (DDDch and DCDch) for each individual registration in the pig sector in Switzerland were recently determined and some theoretically discrepancies between Swiss and European values have been described in a prior study (20).

The aim of this study was to investigate the outcome of calculated antimicrobial consumption in the field based on either individual, Swiss values (DDDch/DCDch) or average, European values (DDDvet/DCDvet). The impact of using either DDD/DCDch or DDD/DCDvet values were tested for different age groups, administration routes and antimicrobial classes. Moreover, the impact of using either DDD/DCDch or DDD/DCDvet for evaluation of antimicrobial use on the study farms was considered, as well as differences in antimicrobial usage by farm type.

The questions behind all these investigations were: Will an AMU monitoring system based on either Swiss or European definitions lead to comparable results in the field or not? And for which age groups, administration routes and antimicrobial classes can the most frequent AMU be observed in the pig sector of Switzerland?

MATERIALS AND METHODS

Data Collection

In cooperation with the Swiss Swine Health Service (SSHS), data from 227 Swiss pig farms concerning antimicrobial use in 2015 was collected, thus representing 3.3% of all pig farms in Switzerland in 2015 (21). All 227 farms joined a nationwide voluntary program for pig producers in Switzerland in order to evaluate and improve transparency of AMU on their farms. Only farms with a complete documentation of antimicrobial ingredients purchased in the year 2015 were included in the study. The study farms were required to provide documentation of all veterinary prescriptions of antimicrobials for this year, including exact information about the name and the amount of the used products. Farmers were required to allocate the prescribed antimicrobials to four different groups (sow, finisher pig, weaner, and piglet). The documentation had to be reported once every 3 months during the year. In addition to AMU records, numbers of pigs kept (sows) or produced annually (all other age groups) and the type of farm were also documented. Overall 96 piglet-producing farms, 42 farrow-to-finish pig farms and 89 finishing farms housing a total of 328,909 piglets, 292,298 weaners, 179,144 finishing pigs and 11,710 sows were included in the study. The number of sows were representing 9.5% of all sows kept in Switzerland, which were notified in 2015 (21). The mean farm size was 85 sows with 2,383 produced piglets and 2,108 produced weaners in the year 2015, including the data of all piglet-producing and farrow-to-finish farms. The mean of the produced finishing pigs was 1,303, combined the data of the farrow-finish farms and the finishing farm, respectively. A piglet-producing farm housing at least 30% piglets from birth until time of slaughter was considered as farrow-to-finish farm.

AMU Quantification

In order to quantify AMU, the amount of prescribed antimicrobial ingredient during the year 2015 of all participating farms was divided by the defined doses (DDD/DCDvet or DDD/DCDch) of the corresponding antimicrobial classes multiplied by the standard weights of the different age groups as defined by the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) (piglets: 4 kg; weaners: 12 kg; finisher pig: 50 kg and sow: 220 kg) (22).

$$Number\ of\ Defined\ Doses = \frac{total\ amount\ of\ prescribed\ antimicrobial\ ingredient\ (mg)}{DDDvet\ or\ DDDch\ or\ DCDvet\ or\ DCDch\ \left(\frac{mg}{kg}\right) \times standard\ weight\ of\ age\ group\ (kg)}$$

The recently published, national defined daily and course doses for the pig sector in Switzerland were drawn up in accordance with the principles of the EMA (19). In order to establish DDDch and DCDch, the required information on dosage and treatment duration was generally taken from the product approvals which are summarized in the

TABLE 1 | Total and relative antimicrobial use (AMU) on 227 Swiss pig farms in the year 2015.

	Amount of active ingredient in kg (percent)	DDDch[a] (percent)	DDDvet[b] (percent)	Ratio	DCDch[c] (percent)	DCDvet[d] (percent)	Ratio	treatment duration ch	treatment duration vet	ratio
Overall Result	421	1,805,494	1,456,771	−19.3%	433,678	303,913	**−29.9%**	3.7	4.0	8.1%
AGE GROUP										
Piglets	15 (3.6%)	473,922 (26.3%)	428,546 (29.4%)	−9.6%	230,237 (53.1%)	132,433 (43.6%)	**−42.5%**	2.9	3.5	**20.7%**
Weaners	208 (49.4%)	1,143,175 (63.3%)	878,525 (60.3%)	**−23.2%**	151,483 (34.9%)	136,136 (44.8%)	−10.1%	5.6	5.0	−10.7%
Finisher pigs	138 (32.8%)	159,719 (8.8%)	122,493 (8.4%)	**−23.3%**	40,894 (9.4%)	27,566 (9.1%)	**−32.6%**	3.7	4.0	8.1%
Sows	60 (14.2%)	28,678 (1.6%)	27,207 (1.9%)	−5.1%	11,064 (2.5%)	7,778 (2.6%)	**−29.7%**	2.9	3.6	**24.1%**
ADMINISTRATION ROUTE										
Oral	0,1 (0.0%)	13,856 (0.7%)	10,080 (0.7%)	**−27.2%**	4,435 (1.0%)	2,432 (0.8%)	**−45.2%**	3.3	4.2	**27.3%**
Injection	114 (27.1%)	620,458 (34.4%)	579,947 (39.8%)	−6.5%	309,277 (71.3%)	182,839 (60.2%)	**−40.9%**	2.7	3.4	**25.9%**
Premix	307 (72.9%)	1,171,180 (64.9%)	866,744 (59.5%)	**−26.0%**	119,966 (27.7%)	118,642 (39.0%)	−1.1%	9.9	7.6	**−23.2%**

AMU is measured as active ingredient and by Swiss and European defined dosage grouped by age category and administration route. The ratio between the number of treatment days and treatments, as well as the overall observed mean treatment durations based on Swiss doses or the doses of the European Medicine Agency (EMA) was calculated. Numbers in bold shown a ratio > ±20%.

[a]DDDch: Number of treatment days based on Swiss Defined Daily Doses.

[b]DDDvet: Number of treatment days based on Defined Daily Doses of the European Medicine Agency (EMA).

[c]DCDch: Number of treatments based on Swiss Defined Course Doses.

[d]DCDvet: Number of treatments based on Defined Course Doses of the European Medicine Agency (EMA).

Swiss Veterinary Medicines Compendium (23). The detailed procedure of defining the national doses and all values for DDDch and DCDch is accessible as **Supplementary Data**. A product and the farm using it were excluded from the study when corresponding DDD/DCDvet values had not been published by the EMA either for the specific antimicrobial ingredient or a comparable antimicrobial ingredient, a given combination of substances or a specific administration route.

The number of defined doses and the amount of prescribed antimicrobial ingredient were calculated in total, for the different age groups, different administration routes (injection, oral, and premix) and for all antimicrobial classes. The term premix included all antimicrobial ingredients to be administered via the feed and/or water. By dividing the results using DDD/DCDvet by those based on DDD/DCDch, differences of Swiss or European definitions were investigated for the calculated AMU. The results of this calculations were termed ratio. A positive ratio with results > 0 indicated a higher number of estimated treatment days or treatments could be observed when using the European definitions DDD vet or DCDvet. In addition, the overall observed mean treatment durations given by the Swiss or European defined values were compared in the same way.

For the evaluation of the AMU at the farm level and in order to compare the consumption on different farms, the number of kept (sows) or produced pigs (other age groups) in the year 2015 were taken into account. The amount of prescribed antimicrobial ingredients was divided by the different defined doses, the standard weights and the number of pigs for each age group. If necessary, the results of the different age groups were summarized together and the number of Defined Doses per farm

TABLE 2 | Total antimicrobial use (AMU) measured as active ingredient and by Swiss and European defined dosage grouped by different antimicrobial classes.

Antimicrobial classes	Amount of active ingredient in kg	DDDch[a]	DDDvet[b]	DCDch[c]	DCDvet[d]
Aminoglycosides	25.7	67,273	59,973	20,918	15,255
Amphenicols	0.03	44	69	22	22
Cephalosporins	0.3	2,200	2,299	733	636
Fluoroquinolones	6.0	171,518	127,880	57,173	39,064
Lincosamides	0.7	26,217	20,456	2,777	2,997
Macrolides	21.4	293,108	120,006	33,286	15,148
Penicillins	77.8	385,507	388,221	229,006	120,394
Pleuromutilins	4.0	14,388	11,289	1,188	1,623
Polypeptides	26.0	335,498	402,708	33,687	43,006
Pyrimidines	2.1	6,613	6,252	1,653	1,705
Sulfonamides	144.1	228,817	98,192	23,946	30,848
Tetracyclins	113.1	274,311	219,426	29,289	33,215

[a]DDDch: Number of treatment days based on Swiss Defined Daily Doses.

[b]DDDvet: Number of treatment days based on Defined Daily Doses of the European Medicine Agency (EMA).

[c]DCDch: Number of treatments based on Swiss Defined Course Doses.

[d]DCDvet: Number of treatments based on Defined Course Doses of the European Medicine Agency (EMA).

was calculated.

$$Number\ of\ Defined\ Doses\ per\ farm = \frac{total\ amount\ of\ prescribed\ antimicrobial\ ingredient\ per\ farm\ and\ age\ group\ (mg)}{DDDvet\ or\ DDDch\ or\ DCDvet\ or\ DCDch\ \left(\frac{mg}{kg}\right) \times standard\ weight\ of\ age\ group\ (kg) \times number\ of\ pigs\ per\ farm\ and\ age\ group}$$

Data Processing and Statistical Analysis

The preparation of all operating farm data and the calculation of the number of defined doses was carried out using Microsoft Excel 2011 (Microsoft, Redmond, WA, USA). The statistical analysis and preparation of graphs to visualize the results was performed with R (https://cran.r-project.org). Differences between the tested groups having a $P \leq 0.05$ were considered statistically significant. The data for calculated AMU on farm level was tested for normality by the Shapiro-Wilk test. The association of Swiss and European dosages for a possible AMU monitoring system on the farms was evaluated using scatterplots and correlation analysis performed by Spearman's rho test. The differences between the various farm structures were investigated using the Kruskal-Wallis test for independent samples and *post hoc* pairwise analysis (Bonferroni correction).

RESULTS

AMU Quantification per Age Group and Administration Route

In this study, the AMU was calculated at 1,805,494 DDDch and 433,678 DCDch when based on Swiss values, compared to 1,456,771 DDDvet (−19.3% ratio) and 303,913 DCDvet (−29.9% ratio) based on European defined doses (**Table 1**). The mean treatment duration was 3.7 days based on Swiss values and 4.0 days based on European values. The largest fraction of DDD was calculated for weaners, regardless of Swiss DDDch (64.4%) or European DDDvet (60.3%), whereas for DCDs based on Swiss definitions, piglets represented the major part of the treatments (53.1%). Based on European definitions most calculated course doses were observed for weaners (44.8%). Ratios of more than 20% between the calculated numbers of DDD/DCDch and

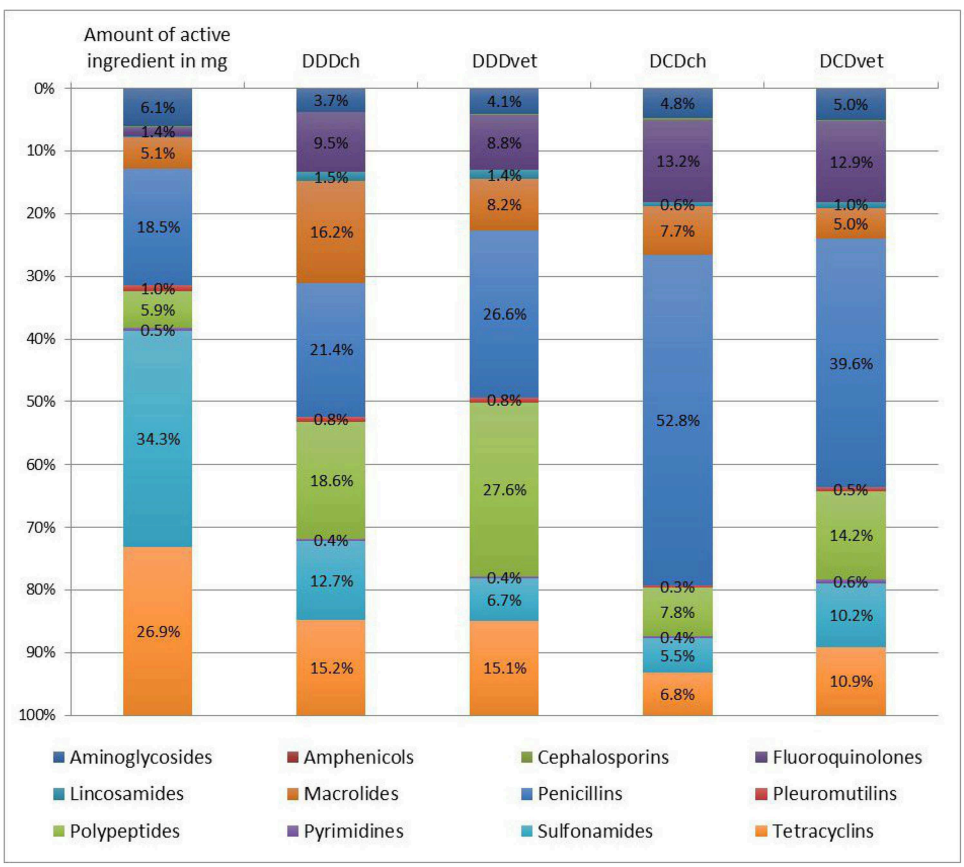

FIGURE 1 | Relative distribution of antimicrobial use (AMU) between different antimicrobial classes measured either as the amount of active ingredient or as the number of defined daily doses (DDD) or defined course doses (DCD), respectively. DDD and DCD were calculated with Swiss values (DDDch and DCDch) or European values (DDDvet or DCDvet) published by the European Medicine Agency (EMA). (Amphenicols and cephalosporins as well as lincosamides are not inscribed due to the low values).

DDD/DCDvet, respectively could be observed for the number of DDDch/vet of weaners and finisher pigs and for DCDch/vet of piglets, finisher pigs, and sows. The largest quantity of active ingredients was given to the groups of weaners (49.4%), followed by finisher pigs (32.7%), sows (14.2%), and piglets (3.6%). When investigating the different administration routes by the number of defined doses, premixes represented the largest proportion calculated by DDDch/vet (64.9/59.5%) in contrast to injectable products when calculating the number of DCDch/vet (71.3/60.2%). Relative differences of more than ±20% could be observed for oral and premix treatments when calculated in DDDch and DDDvet, respectively, and for oral and parenteral treatments when calculated in DCDch or DCDvet. The treatment duration was longer when the calculation was based on DDD/DCDvet compared to DDD/DCDch, except for treatments of weaners in general and for treatments with premixes.

AMU Quantification per Antimicrobial Classes

The amount of active ingredient and the calculated numbers of defined doses for different antimicrobial classes were summarized in **Table 2** and the relative distribution was visualized in **Figure 1**. Considering the amount of active ingredient used, the classes of sulfonamides (144,086,000 mg/34.3%), tetracyclines (113,122,600 mg/26.9%), and penicillins (77,788,850 mg/18.5%) represented the largest proportion of the total usage, whereas when using defined daily doses, penicillins (DDDch: 385,507/21.4%; DDDvet: 388,221/26.6%) and polypeptides (DDDch: 335,498/18.6%; DDDvet: 402,708/27.6%) were the most frequent. Macrolides were observed to represent 16.2% of the total usage (293,108 treatment days) calculated in DDDch. Penicillins (DCDch: 229,006/52.8%; DCDvet: 120,394/39.6%) and fluroquinolones (DCDch: 57,173/13.2%; DCDvet: 39,064/12.9%) were common for the number of total treatments, as well as polypeptides (DCDvet: 43,006/14.2%) for calculations based on the European values. The percentage of fluoroquinolones in total AMU was 1.4% when considering the amount in mg, compared to 8.8 and 13.2% when calculating DDDvet and DCDch, respectively.

A more detailed, combined consideration of age groups, administration routes, and antimicrobial class data shows that injection was the most frequent administration route for piglets independent of the method used for calculation, and that within this group penicillins and fluoroquinolones were the most frequently used antimicrobials (**Table 3**). The use of premixes was the most frequently used administration route for weaners independent of the indicator used and polypeptides were most frequently used when considering the number of defined daily doses. For the number of calculated doses based on DDD/DCDch, frequent use of macrolides was notable in the premixes given to weaners whereas sulfonamides and tetracyclines were more frequently used when the calculation was based on DDD/DCDvet. In terms of the finisher pig group, injection and premixes were observed with similar frequencies for administration routes, when either daily doses or course doses were the basis of the calculation. Oral administration of premixes was the most common administration route when calculating AMU based on defined doses. Contrastingly, when calculating in course doses, injections represented the largest proportion of treatments. Penicillins and aminoglycosides were frequently used injections for finisher pigs and tetracyclines were the most commonly used antimicrobial class given as premix. As was the case in weaners, macrolides represented a considerable proportion of treatments based on DDDch as well as DCDch. In sows, most antimicrobials were given by injections and within this group, most of the antimicrobials administered belonged to the antimicrobial classes of penicillins and fluoroquinolones. The most frequently administered antimicrobial class provided as a premix was the class of penicillins. An administration of oral antimicrobials without feed or water was only observed for fluoroquinolones and polypeptides in piglets and on only two farms with a small amount in weaners.

AMU Monitoring on Farm Level

Each dataset was tested for normality by Shapiro-Wilk test and for all datasets, independent of Swiss or European measuring method or type of farm, the null hypothesis was rejected (each $P < 0.001$).

The scatterplot of calculated defined daily doses (DDD) and defined course doses (DCD), analyzing the association between Swiss (ch) and European (vet) definitions, is given in **Figure 2**. As shown, both the calculated number of daily doses and the calculated number of course doses showed a positive correlation between results on the farm level by Spearman's rho test.

Consideration of structure of the various farms pointed to a higher amount of calculated AMU per farm and per year on farrow-to-finishing farms and piglet-producing farms compared to finishing farms for all Swiss or European values of defined doses ($P < 0.001$) by Kruskal-Wallis-test and subsequent *post hoc* pairwise analysis (**Table 4**, **Figure 3**). In terms of calculated DDDch-numbers the median values were 4.40, 4.88, and 0.27 for farrow-to-finishing, piglet-producing and finishing farms, respectively. No significant difference between the farrow-to-finishing farms and the piglet-producers was observed for any of the used values.

DISCUSSION

This study shows that although evaluating AMU for the pig sector at the farm level based either on Swiss or European defined doses leads to similar results with a positive correlated association, there were still deviations in detail, i.e., in the assessment of the different active substance classes, different administration routes and various age groups. A possible on farm AMU monitoring system will arrive at similar conclusions and farms with low or high AMU consumption will be similarly assessed using both methods. Since the Swiss definitions are based on individual national approvals in comparison to the average EMA definitions collected from nine countries, the Swiss definitions seem more robust for a national evaluation of active substance classes, administration routes and age groups.

The challenge of collecting adequate information on AMU in the field is well-known and described in the literature (15). Since

TABLE 3 | Distribution of antimicrobial use (AMU) per different age categories, administration routes and antimicrobial classes measured as active ingredient and by Swiss and European defined dosage.

Age group	Administration route	Antimicrobial classes	Amount of active ingredient in mg		DDDch[a] (*n*)	(%)	DDDvet[b] (*n*)	(%)	DCDch[c] (*n*)	(%)	DDDvet[d] (*n*)	(%)
Piglets			15,117,075		473,922		428,546		230,237		132,433	
	Oral		118,250	0.8%	13,833	2.9%	10,065	2.3%	4,428	1.9%	2,428	1.8%
		Fluoroquinolones	83,050	70.2%	12,458	90.1%	8,305	82.5%	4,153	93.8%	2,076	85.5%
		Polypeptides	35,200	29.8%	1,375	9.9%	1,760	17.5%	275	6.2%	352	14.5%
	Injection		14,764,825	**97.7%**	450,340	**95.0%**	406,781	**94.9%**	224,835	**97.7%**	128,760	**97.2%**
		Aminoglycosides	2,662,850	18.0%	39,802	8.8%	36,445	9.0%	13,419	6.0%	9,661	7.5%
		Cephalosporins	7,875	0.1%	880	0.2%	916	0.2%	293	0.1%	259	0.2%
		Fluoroquinolones	1,281,725	8.7%	129,323	**28.7%**	95,830	**23.6%**	43,108	**19.2%**	29,632	**23.0%**
		Lincosamides	297,500	2.0%	14,875	3.3%	9,535	2.3%	2,125	0.9%	2,010	1.6%
		Macrolides	98,000	0.7%	2,450	0.5%	1,885	0.5%	817	0.4%	471	0.4%
		Penicillins	10,118,275	**68.5%**	257,065	**57.1%**	253,251	**62.3%**	163,096	**72.5%**	83,993	**65.2%**
		Pleuromutilins	20,000	0.1%	400	0.1%	417	0.1%	133	0.1%	227	0.2%
		Pyrimidines	22,000	0.1%	1,857	0.4%	1,833	0.5%	464	0.2%	500	0.4%
		Sulfonamides	110,000	0.7%	1,857	0.4%	1,782	0.4%	464	0.2%	480	0.4%
		Tetracyclins	146,600	1.0%	1,833	0.4%	4,887	1.2%	916	0.4%	1,527	1.2%
	Premix		234,000	1.5%	9,750	2.1%	11,700	2.7%	975	0.4%	1,245	0.9%
		Polypeptides	234,000	100.0%	9,750	100.0%	11,700	100.0%	975	100.0%	1,245	100.0%
Weaners			207,658,150		1,143,175		878,525		151,483		136,136	
	Oral		450	0.0%	23	0.0%	15	0.0%	8	0.0%	4	0.0%
		Fluoroquinolones	450	100.0%	23	100.0%	15	100.0%	8	100.0%	4	100.0%
	Injection		8,654,000	4.2%	82,799	7.2%	89,073	10.1%	42,440	28.0%	28,684	21.1%
		Aminoglycosides	755,300	8.7%	5,946	7.2%	4,621	5.2%	1,790	4.2%	1,252	4.4%
		Cephalosporins	12,500	0.1%	486	0.6%	508	0.6%	162	0.4%	141	0.5%
		Fluoroquinolones	567,400	6.6%	21,078	25.5%	16,860	18.9%	7,026	16.6%	5,237	18.3%
		Lincosamides	62,500	0.7%	1,042	1.3%	668	0.7%	149	0.4%	141	0.5%
		Macrolides	100,000	1.2%	833	1.0%	641	0.7%	278	0.7%	160	0.6%
		Penicillins	5,955,400	68.8%	46,766	56.5%	52,806	59.3%	30,355	71.5%	17,753	61.9%
		Pleuromutilins	10,000	0.1%	67	0.1%	69	0.1%	22	0.1%	38	0.1%
		Pyrimidines	36,400	0.4%	1,264	1.5%	1,011	1.1%	316	0.7%	276	1.0%
		Sulfonamides	182,000	2.1%	1,264	1.5%	1,083	1.2%	316	0.7%	310	1.1%
		Tetracyclins	972,500	11.2%	4,052	4.9%	10,806	12.1%	2,026	4.8%	3,377	11.8%
	Premix		199,003,700	**95.8%**	1,060,353	**92.8%**	789,437	**89.9%**	109,036	**72.0%**	107,448	**78.9%**
		Aminoglycosides	268,400	0.1%	10,167	1.0%	6,578	0.8%	484	0.4%	828	0.8%
		Lincosamides	268,400	0.1%	10,167	1.0%	10,167	1.3%	484	0.4%	828	0.8%
		Macrolides	15,594,000	7.8%	264,722	**25.0%**	108,292	13.7%	29,222	**26.8%**	13,260	12.3%
		Penicillins	5,613,500	2.8%	22,598	2.1%	27,517	3.5%	4,520	4.1%	2,613	2.4%
		Pleuromutilins	400,000	0.2%	4,444	0.4%	3,436	0.4%	317	0.3%	412	0.4%

(Continued)

TABLE 3 | Continued

Age group	Administration route	Antimicrobial classes	Amount of active ingredient in mg		DDDch[a] (*n*)	(%)	DDDvet[b] (*n*)	(%)	DCDch[c] (*n*)	(%)	DDDvet[d] (*n*)	(%)
		Polypeptides	22,934,400	11.5%	318,533	**30.0%**	382,240	**48.4%**	31,853	**29.2%**	40,664	**37.8%**
		Sulfonamides	91,210,000	45.8%	200,556	18.9%	82,618	10.5%	20,056	**18.4%**	26,120	**24.3%**
		Tetracyclins	62,715,000	31.5%	229,167	21.6%	168,589	21.4%	22,099	**20.3%**	22,723	**21.1%**
Finisher pigs			137,539,345		159,719		122,493		40,894		27,566	
	Injection		36,317,845	26.4%	59,832	37.5%	58,296	47.6%	31,176	**76.2%**	17,751	**64.4%**
		Aminoglycosides	9,080,625	25.0%	8,386	**14.0%**	9,221	**15.8%**	3,872	**12.4%**	2,627	**14.8%**
		Amphenicols	32,700	0.1%	44	0.1%	69	0.1%	22	0.1%	22	0.1%
		Cephalosporins	31,500	0.1%	315	0.5%	332	0.6%	105	0.3%	89	0.5%
		Fluoroquinolones	108,125	0.3%	915	1.5%	713	1.2%	305	1.0%	220	1.2%
		Lincosamides	30,000	0.1%	120	0.2%	77	0.1%	17	0.1%	16	0.1%
		Macrolides	653,600	1.8%	1,307	2.2%	1,006	1.7%	436	1.4%	251	1.4%
		Penicillins	24,298,495	**66.9%**	46,202	**77.2%**	41,541	**71.3%**	25,360	**81.3%**	12,839	**72.3%**
		Pleuromutilins	90,000	0.2%	144	0.2%	150	0.3%	48	0.2%	82	0.5%
		Pyrimidines	58,000	0.2%	377	0.6%	387	0.7%	94	0.3%	105	0.6%
		Sulfonamides	290,000	0.8%	377	0.6%	414	0.7%	94	0.3%	129	0.7%
		Tetracyclins	1,644,800	4.5%	1,645	2.7%	4,386	7.5%	822	2.6%	1,371	7.7%
	Premix		101,221,500	73.6%	99,887	**62.5%**	64,197	**52.4%**	9,718	23.8%	9,815	35.6%
		Macrolides	4,897,000	4.8%	23,767	**23.8%**	8,162	12.7%	2,525	**26.0%**	999	10.2%
		Penicillins	1,950,000	1.9%	1,917	1.9%	2,294	3.6%	383	3.9%	218	2.2%
		Pleuromutilins	350,0000	3.5%	9,333	9.3%	7,216	11.2%	667	6.9%	864	8.8%
		Polypeptides	1,752,000	1.7%	5,840	5.8%	7,008	10.9%	584	6.0%	746	7.6%
		Sulfonamides	42,145,000	41.6%	21,567	21.6%	9,162	14.3%	2,210	22.7%	2,897	29.5%
		Tetracyclins	46,977,500	46.4%	37,464	**37.5%**	30,355	**47.3%**	3,349	**34.5%**	4,091	**41.7%**
Sows			59,835,480		28,678		27,207		11,064		7,778	
	Injection		54,560,980	**91.2%**	27,489	**95.9%**	25,797	**94.8%**	10,826	**97.8%**	7,644	**98.3%**
		Aminoglycosides	12,929,300	23.7%	2,972	10.8%	3,108	12.0%	1,352	12.5%	885	11.6%
		Cephalosporins	236,525	0.4%	519	1.9%	544	2.1%	173	1.6%	148	1.9%
		Fluoroquinolones	3,930,475	7.2%	7,722	**28.1%**	6,157	**23.9%**	2,574	**23.8%**	1,895	**24.8%**
		Lincosamides	15,000	0.0%	14	0.0%	9	0.0%	2	0.0%	2	0.0%
		Macrolides	62,000	0.1%	28	0.1%	22	0.1%	9	0.1%	5	0.1%
		Penicillins	24,578,680	**45.0%**	9,769	**35.5%**	9,401	**36.4%**	5,054	**46.7%**	2,844	**37.2%**
		Pyrimidines	1,993,800	3.7%	3,116	11.3%	3,021	11.7%	779	7.2%	824	10.8%
		Sulfonamides	10,149,000	18.6%	3,197	11.6%	3,133	12.1%	806	7.4%	914	12.0%
		Tetracyclins	666,200	1.2%	151	0.6%	404	1.6%	76	0.7%	126	1.7%
	Premix		5,274,500	8.8%	1,189	4.1%	1,410	5.2%	238	2.2%	134	1.7%
		Penicillins	5,274,500	100.0%	1,189	100.0%	1,410	100.0%	238	100.0%	134	100.0%

Numbers in bold are mentioned in the results part of the study.

[a] *DDDch: Number of treatment days based on Swiss Defined Daily Doses.*

[b] *DDDvet: Number of treatment days based on Defined Daily Doses of the European Medicine Agency (EMA).*

[c] *DCDch: Number of treatments based on Swiss Defined Course Doses.*

[d] *DCDvet: Number of treatments based on Defined Course Doses of the European Medicine Agency (EMA).*

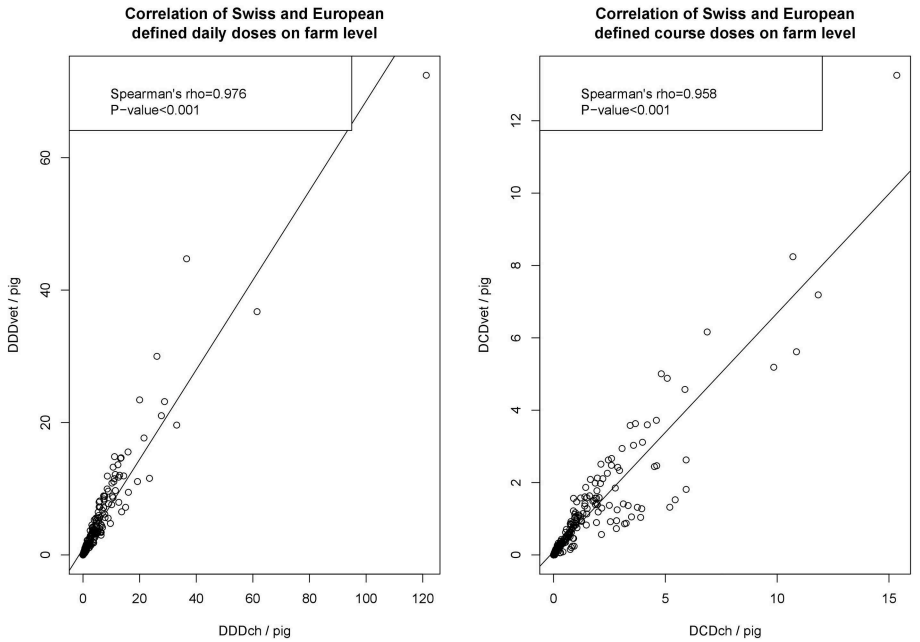

FIGURE 2 | Scatterplots of defined daily doses (DDD) and defined course doses (DCD) at the farm level calculated either by Swiss values (DDDch/farm and DCDch/farm). Each dataset was tested for normality by Shapiro-Wilk test and for all datasets the null hypothesis was rejected (each $P < 0.001$). So non-normal distributed data was concluded and the correlation was investigated by Spearman's rho test.

TABLE 4 | Median values of the defined daily doses (DDD) and defined course doses (DCD) based on the number of Switzerland (DDDch/farm and DCDch/farm) and the European Medicine Agency (DDDvet/farm and DCDvet/farm) for the different type of farms (farrow-to-finish farm, finishing farm and piglet-producing farm).

	DDDch/farm	DDDvet/farm	DCDch/farm	DCDvet/farm
1) Farrow-to-finish farm	4.40 (0.67–16.02)	3.63 (0.83–15.46)	1.43 (0.27–4.48)	0.98 (0.239–3.63)
2) Finishing farm	0.27* (0–3.82)	0.26* (0–2.75)	0.08* (0–0.70)	0.077* (0–0.50)
3) Piglet-producing farm	4.88 (0.96–12.45)	3.99 (1.04–12.04)	1.22 (0.29–4.61)	1.05 (0.26–2.65)

*10 and 90% percentiles are given in brackets. Each dataset was tested for normality by Shapiro-Wilk tests and for all datasets the null hypothesis was rejected (each $P < 0.001$). So non-normal distributed data was concluded. By performing Kruskal-Wallis test for independent samples and post hoc pairwise analysis (Bonferroni correction) significant differences between finishing farm and farrow-to-finish-farm respectively, piglet-producing farm could be observed (each $P < 0.001$). No significant differences between farrow-to-finishing farm and piglet-producing farm could be observed (each $P > 0.05$). *$P < 0.001$ (to 1 and 3).*

the participation in the present study and supply of data was voluntary, some bias cannot be completely ruled out due to the fact that knowledge and motivation of farmers have an influence on AMU (24). We consider the coverage of the study population to be adequate for our study goals with 3.3% of all Swiss pig farms and 9.5% of all sows, and it allows to deduce that especially larger farms seemed to be more motivated to participate in the study.

Since the data underlying this study did not include a record about the length of pigs' stay in the farrowing unit, the nursery unit and the fattening unit, it is not feasible to make an exact evaluation of how many theoretical treatment days or treatments would be possible in the life span of a pig, as calculated by Timmermann et al. (25). However, the calculation behind the number of dosages on farm level is based on the population of animals present or produced during 1 year and this makes it comparable to other systems using defined doses to estimate AMU per farm in livestock (11–13).

Since the present study is based on calculations from prescribed amounts, the exact amounts of antimicrobials used by the farmer cannot be assessed and overdosing as well as underdosing could bias the results and the study only allows a statistical estimation of the probable AMU.

Another aspect of this study which is shown in **Table 1** is the different evaluation of monitoring systems based either on the measurement of the amount of active ingredient or on the measurement of application equivalents such as defined doses: due to the lower standard weight of piglets, a considerable number of defined treatments can be performed with an amount of antimicrobial suitable for a single treatment of just one sow. As a consequence, the observed amount of active ingredients for e.g., piglets was low whereas the number of calculated doses was high. This is in line with prior studies (26) and EMA advice cautioning that differences in dosing between species and substances must be taken into account when using DDD and DCD values (19).

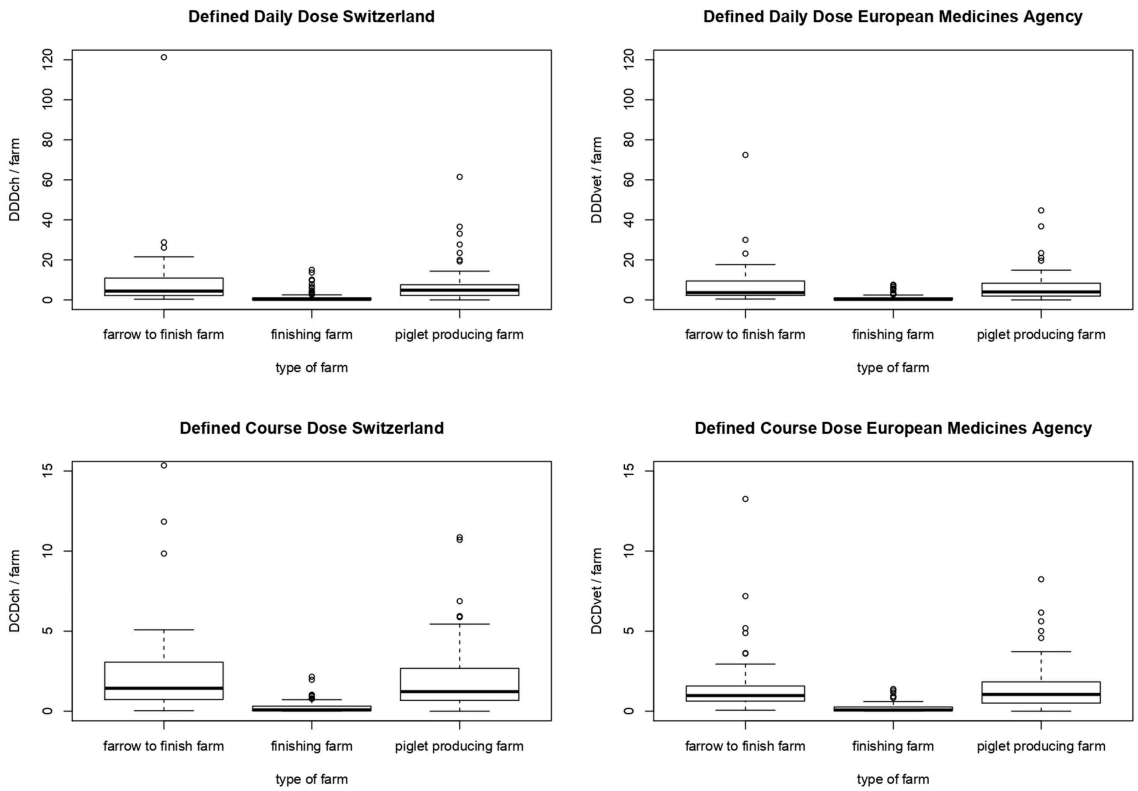

FIGURE 3 | Comparison of antimicrobial use in different types of farms (farrow-to-finish farms, finishing farms and piglet-producing farms) measured by the number of defined daily doses (DDD) and defined course doses (DCD) per farm based on the values of Switzerland (DDDch/farm and DCDch/farm) and the European Medicine Agency (DDDvet/farm and DCDvet/farm). Each dataset was tested for normality by Shapiro-Wilk test and for all datasets the null hypothesis was rejected (each $P < 0.001$). So non-normal distributed data was concluded. By performing Kruskal-Wallis test for independent samples and *post hoc* pairwise analysis (Bonferroni correction) significant differences between finishing farm and farrow-to-finish-farm respectively, piglet-producing farm could be observed (each $P < 0.001$). No significant differences between farrow-to-finishing farm and piglet-producing farm could be observed (each $P > 0.05$).

In general, a low value for a defined dose results in a higher number of calculated or estimated doses in a population (17). This explains some differences between the number of DDDch or DCDch on the one side and DDDvet and DCDvet on the other side. For example, macrolides showed a difference in calculated use depending on whether Swiss or European definitions were chosen. As a previous study showed, there are six Swiss premix products containing the macrolide tylosin with much lower defined daily and course doses compared to the values of the EMA (20), thus explaining the relatively high number of DDDch and DCDch in this category. This general understanding can also be used to explain the results in **Table 1**. All groups with a high ratio between the calculation based on Swiss or European definitions come by a frequent use with approvals whose DDDch and DCDch values differ strongly from the DDDvet and DCDvet values.

In accordance with a recently published study, the animal groups with the highest numbers of treatment days and total number of treatments observed were weaners (DDDch, DDDvet, and DCDvet) and piglets (DCDch) (27). These groups are most susceptible to bacterial infections and, at least for the weaners, frequent group therapies at weaning can be assumed, which is reflected in the high proportion of treatments with premixes, as described by other studies (28). This assumption is also underlined by the fact, that a longer Swiss treatment duration could be observed only for weaned piglets and for premixes and a relationship between both findings could be hypothesized. Thus, young age groups should already be considered in terms of resistance prevention and the use of group therapies by premixes in feed in these groups should be critically re-evaluated (29).

Furthermore, when calculating the number of DDDs, relatively high use could be observed for premixes and in contrast, a relatively high total number of course doses could be observed for injections in this study. This can be explained by the comparison of treatment durations between injections and premixes, since the number of calculated course doses decreases with the increase in treatment duration of the premixes. Previous publications confirmed the high proportion of premixes used in the pig sector in Switzerland (30, 31). An increased risk of development of resistance for specific active substances and bacteria is documented by this administration route (32). Thus, group therapies should be reduced to the necessary minimum.

A relatively high proportion of treatments with Highest Priority Critically Important Antimicrobials (HPCIAs) could be observed in this study (e.g., 44.8% of DDDvet's). These findings are comparable to results recently published from the EFFORT consortium (27), but varying from results of a previous study, where a lower AMU quantification of HPCIAs for pigs in Switzerland based on total amount of given active ingredient was observed (33). Due to the documented spread of resistance genes e.g., against fluoroquinolones in the pig sector (34), every use of these substances should be of concern and further research investigating restriction of indications and potential reductions in usage is needed.

The results from the different farm types show again that the younger age groups are most frequently treated. Both, farrow-to-finishing farm as well as piglet-producing farms in contrast to the finishing farms keep the high consumption age groups of piglets and weaners. This could explain the significant difference. Due to the small number of calculated defined doses of finisher pigs, no significant difference between farrow-to-finishing and piglet-producing farms was observed.

In order to gain a better understanding of the differences between these individual farms, further studies are needed to examine the role of the farmer (23) as well as AMU quantification and performance data (35).

CONCLUSION

In summary, this study demonstrated a general association of the AMU systems at the farm level, nevertheless, differences were seen in detail according to whether the calculation was based on individual Swiss or average European values. The benefit of the European values for internationally comparative AMU monitoring is undisputed, but for a detailed evaluation, Swiss definitions could be more accurate as they are based on the specific approvals of the country. This must be considered in order to understand international AMU comparisons in the future. The study also highlighted the need to further evaluation for the use of HPCIAs.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

Since this was only data that had no influence on the actual treatment of the animals, an animal welfare permit was not required. No manipulations or something similar were carried out on any animals.

AUTHOR CONTRIBUTIONS

DK, XS, CM, and TE contributed conception and design of the study. TE organized the database and performed the statistical analysis. CM is responsible for the correctness of the pharmacological formulations and the part in the manuscript about measurement methodologies. TE wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2019.00240/full#supplementary-material

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ORIGINAL RESEARCH
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Monitoring Antibiotic Usage in German Dairy and Beef Cattle Farms—A Longitudinal Analysis

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It is well-established that antimicrobial use is a major factor for the development of antimicrobial resistance. To analyze the associations between antimicrobial resistance and usage of antimicrobial agents, data from monitoring and surveillance systems are crucial. Within the project VetCAb (Veterinary Consumption of Antibiotics), antibiotic usage data in German livestock is regularly collected and evaluated. Based on a cross-sectional study in 2011, the project was continued as the longitudinal study VetCAb-Sentinel with ongoing participant recruitment and data collection from 2013. The data collection is based on official German application and delivery forms (ADF), voluntarily provided by veterinarians and farmers. In this study the results of antibiotic usage data of dairy cows, dairy calves and beef cattle were described, using a semi-annual treatment frequency, and 95,944 ADF issued between 2011 and 2015 were analyzed. Results show that the median of the treatment frequency in dairy calf and beef cattle holdings slightly decreased from 0.4 to 0.3 and from 0.2 to 0 days, respectively, whereas the median in dairy cow holdings ranged between 1.9 and 2.3 during the observed period. Temporal changes and the effect of the factors "farm size" and "region" on the treatment frequency were investigated, using multiple linear mixed and logistic regression models. Generally, the factor "time" has a statistically significant impact on the treatment frequency in all production types. In addition, a temporal trend test over the first six half-years shows that an increasing linear trend can be stated in dairy cows and dairy calves ($p = 0.017$; $p = 0.004$, respectively). If the time-period is extended to all eight half-years under study, this turns into a quadratic effect (dairy cows: $p = 0.006$; dairy calves: $p < 0.001$). In dairy calves and beef cattle the factor "farm size" also has a statistically significant impact. The factor "region," in contrast, shows no statistically significant impact at all. Compared to other livestock populations in Germany, the use of antimicrobials in dairy cows, dairy calves, and beef cattle appears to be low, but varies across several associated factors. Considering these effects, it is recommended that the size of dairy calf and beef cattle holdings is regularly considered in the evaluation of antimicrobial usage data over time.

Keywords: monitoring of antimicrobial consumption, treatment frequency, regression modeling, dairy cows, dairy calves, beef cattle

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INTRODUCTION

The impact of the use of antibiotics on antimicrobial resistance (AMR) in food-producing animals has been the subject of increasing public, scientific and political debate in recent years. It is well-known that the development of resistance is related to some extent to the antibiotic use (1, 2). Therefore, for regular evaluation of these associations and for interpretation of resistance patterns and trends, among others, detailed information about antibiotic consumption is needed (3, 4). At the EU level, Directive 2003/99/EC requires the member states to carry out a monitoring of AMR in zoonotic agents and commensal bacteria (5). In Germany, since 2011, the amount of veterinary medicinal products containing antimicrobials delivered to veterinarians by pharmaceutical companies and wholesalers is documented in a central information system and evaluated annually by the Federal Office of Consumer Protection and Food Safety (BVL) (6). Results show that the amount of antibiotics have been reduced by more than half by 2015 (7). These data are also reported to the European Surveillance of Veterinary Antimicrobial Consumption project (ESVAC), which was launched in 2009 by the European Medicines Agency (EMA), following a request by the European Commission to develop an approach for the harmonized collection and evaluating of antimicrobial usage (AMU) sales data in animals in the member states (8).

In April 2014 the 16th amendment of the German Medicinal Products Act (AMG) was introduced, which requires farmers that keep fattening animals to report their usage of applied veterinary medicinal products with antibiotic components on a half yearly basis (9). To comply with legal requirements, the use of medicines in livestock animals per-production type is recorded by farmers and veterinarians directly in one specific national database (Herkunftssicherungs- und Informationssystem für Tiere). There, data are collected separately for each production type of fattening cattle, pigs, chicken and turkey to determine a farm-specific half-year treatment frequency (TF). Based on these, semiannually the BVL determines the median and third quartile of the TF for each of these livestock populations, which is the basis for further actions, such as consulting the veterinarian or writing an action plan to reduce AMU (9).

Monitoring systems that pursue economic or scientific interests were also introduced in Germany. The private company "QS Qualität und Sicherheit GmbH" (QS) offers a benchmark system on farm level in Germany for poultry, pigs, and calves for fattening (10).

In the frame of the scientific projects VetCAb and VetCAb-Sentinel (VetCAb-S), the antibiotic usage at farm level is determined by used quantities and number of applied single doses. In the latter project not only the usage of antibiotics in livestock used for fattening, but also of dairy cows and dairy calves is recorded and evaluated.

The aim of this work is to present the results of data analysis on antibiotic usage in dairy cows, dairy calves and beef cattle in the years 2011, 2013, 2014, and 2015. Moreover, the association between temporal trends and the factors "farm size," "region," and "veterinarian" on the AMU is analyzed.

Abbreviations: ADF, Application and Delivery Form; AMG, Medicinal Products Act ("Arzneimittel-Gesetz"); AMR, Antimicrobial Resistance; AMU, Antimicrobial Usage; BVL, Federal Office of Consumer Protection and Food Safety ("Bundesamt für Verbraucherschutz und Lebensmittelsicherheit"); ESVAC, European Surveillance of Veterinary Antimicrobial, Consumption; IQR, Interquartile Range; (n)DDD, (number of) Defined Daily Dose(s); (n)DDDA$_F$, (number of) Defined Daily Dose(s) Animal at farm level; (n)UDD, (number of) Used Daily Dose(s); TF, Treatment Frequency; QS, Qualität und Sicherheit GmbH; VetCAb-S, Veterinary Consumption of Antibiotics—Sentinel.

MATERIALS AND METHODS

Study Population and Data Collection

Data for 2011 were collected within the pilot phase of the VetCAb project with a cross-sectional approach (11). To ensure a cross-sectional study like study population the data was checked for its representativeness by investigating the demographic characteristics of the participating farms by comparing these with official data of the agricultural statistics (12). Since 2013, the project is continued as a longitudinal study with ongoing participant recruitment, called VetCAb-S (13). The study population was initially recruited as a convenience sample by addressing all veterinarians and farmers by general information in newsletters and the German Veterinary Record ("Deutsches Tierärzteblatt"), which is sent out mandatorily to all veterinarians in Germany. Farmers and veterinarians voluntarily provide AMU data via ADF about the number of animals treated, date and duration of treatment, name and amount of the medicinal product used, indication and application route (14). Information on the number of livestock places, i.e., the animal capacity of the individual farms, is requested separately. After checking completeness and pharmacological plausibility as previously described (15), data are included in the evaluation.

In this survey, three production types are considered: dairy cows, dairy calves and beef cattle. Dairy cows are defined as cows kept for milk production. The group of dairy calves includes calves reared on dairy farms for later use as dairy or beef cattle. The number of livestock locations for dairy calves is not collected directly, it is assumed as the number of livestock locations for dairy cows that are kept on the farm. Beef cattle are defined as cattle from 8 month old, reared for meat production. Because each participating farm can keep one or more production types, the allocation to the respective groups is mainly based on the category given on the ADFs.

Measuring Antibiotic Usage

In order to quantify antibiotic usage, the number of antimicrobial substance applications (number of used daily doses, nUDD) is determined using the records in the database as follows:

$$nUDD = \textit{number of animals treated} \times \textit{number of days treated} \times \textit{number of active ingredients}$$

By means of the TF, the average number of treatments per animal of the observed population within a given time period is

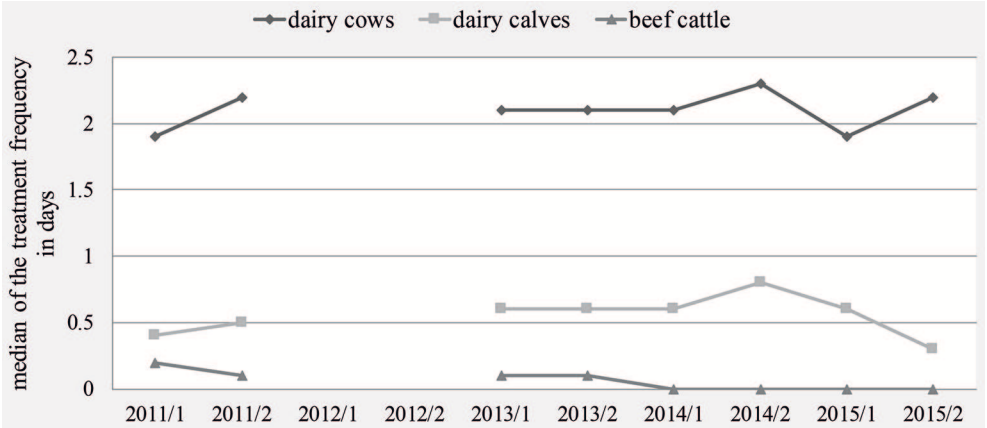

FIGURE 1 | Median of the treatment frequency per half-year for dairy cows, dairy calves and beef cattle.

calculated (16–18):

$$TF = \frac{nUDD}{farm\ size}$$

Following the general rules of the AMG, the measurements for all applications are calculated for each holding per half-year. Treatment of udder diseases and all treatments in the context of dry-cow therapy are included in the evaluations. Each production type kept on a farm within half a year is defined as a holding in the analysis. In the project, the reference population is defined by number of available livestock places per holding. The population under study is herein referred to as the "study collective." When entering the study collective, the number of livestock locations of dairy cows and beef cattle of every farm was recorded. This information serves as a basis for calculating the TF over the entire period.

Statistical Analysis

Two statistical model evaluations were applied. In order to analyse whether there are trends in the development of the TF over time, linear and quadratic trend effects of the TF were calculated with polynomial regression by orthogonal polynomial coefficients within linear models. Due to different sub-trends within the data, the calculations were carried out over two periods, based on the first six and on all eight considered half-years from 2011 to 2015.

In a second approach, the general impacts of the factors "time," "farm size," and "region" on antibiotic usage in dairy cows and dairy calves were considered using multiple generalized linear mixed regression models for calculating a three-way ANOVA with nested subjects, using the TF as the outcome. For this purpose, a right-trimmed data set was used to guarantee robust model estimators, where the top 1% TFs were excluded (19). The same method has already been used on pigs (15). As the antibiotic usage is measured semi-annually, there are eight observations per holding within the analyzed time period. The missing year 2012 leads to different intervals between the regarded time points. A flexible correlation structure between observations of one farm is chosen due to the non-equidistant time points. The estimated covariance parameters showed that covariance between time points 2011–1 and 2011–2 with others are smaller than those of later time points. Therefore, a structure with constant covariance or e.g., auto-regressive structures are not suitable. The choice of the variance structure affects the model estimates of variances and consequently the observed confidence intervals and *p*-values. The factor "farm size" was categorized into three groups by means of the 33- and 66%-percentile of the number of livestock places per holding on the basis of study population in 2011. The cut-offs for dairy cows were 59 and 116, for dairy calves were 55 and 114, and for beef cattle 35 and 60 livestock places, respectively. For data analysis referring to the factor "region," the examined collective of cattle farms is divided into three geographical areas (Middle, Northwest and East Germany) based on agricultural structures in Germany (20). Only a small proportion of participating beef cattle holdings from the eastern region has been documented, therefore these holdings were not considered for evaluation. According to Hemme et al. (15), the impact of the veterinarian on the TF was taken into account as a random effect following a hierarchical model structure (15). Compound-symmetry covariance structure for the modeling of the random veterinary effect was assumed. Impact of the veterinarian random effect was analyzed by using a likelihood ratio chi-square test comparing the full model with the reduced model, thus omitting the hierarchical level. We considered three different regression models for evaluation in terms of transformation due to a skewed distribution of residuals: square root transformation, logarithm transformation after adding 0.1 and logarithm transformation after adding 1. Results were converted to the original scale after retransformation of least-squares means with associated 95% confidence intervals. The residuals of the final models were distributed normally. Due to zero inflated data of beef cattle, an appropriate result regarding the distribution of the residuals could not be achieved when comparing the different transformations. Therefore, no adequate model for the TF could be adapted. Hence, we conducted a multi-factorial mixed logistic regression to model the antibiotic

usage. In the logistic regression, the odds-ratio confidence intervals were calculated to describe the effect of risk factors. The estimation was done applying the Residual Pseudo Likelihood method. The 95% confidence intervals for parameters of interest were reported.

The analyses were performed in SAS, version 9.3 TS level 1M2 (SAS Institute Inc., Cary, NC, United States), using the procedures MIXED and GLIMMIX, respectively, and entailing F-tests to assess the statistical significance of fixed effects. *P*-values below 5% were considered as statistically significant.

RESULTS

Study Population

During the observational period, a total of 95,944 ADFs from participating dairy and beef cattle farms for the years 2011, 2013, 2014, and 2015 were evaluated. Of these, 79,528 ADFs were allotted to dairy cows, 14,424 ADFs to dairy calves and 1,992 ADFs to beef cattle. Due to the two project phases, pilot and sentinel study, a drop in the number of participating dairy cow and dairy calf holdings was evident between 2011 and 2013. Seventeen percent of the dairy cow and calf holdings and 16% of the beef cattle holdings participated throughout the entire period considered. The other part consisted of holdings, which participated in sections, joined the collective later than 2011 or left the collective earlier than 2015. The discrepancy between the analyzed number of cow and dairy calf holdings resulted from the trimmed 1% of the semi-annual TF and disregarded holdings, respectively. At the beginning of the sentinel study in 2013, the number of participating beef cattle holdings could be increased and then kept at a constant level (see **Table 1**). Within the study collective, the following numbers of antibiotic substance prescriptions were made per holding half-yearly in the median: 27 for dairy cows (Interquartile range (IQR) = 12–48 prescriptions per holding), three for dairy calves (IQR = 1–8 prescriptions per holding) and one for beef cattle (IQR = 0–3 prescriptions per holding). Most of these holdings were located in northwest Germany, followed by holdings from the middle and east of Germany.

Antibiotic Usage and Treatment Frequency

Table 1 shows the distribution of the semi-annual TF of dairy cows, dairy calves and beef cattle holdings within the observed time period. In dairy cow holdings, the median of the semi-annual TF was quite constant with minor deviations. In dairy calves, the median of the semi-annual TF increased from 0.4 in the first half-year of 2011 to 0.8 in the second half-year of 2014, before dropping to 0.3 in the second half-year of 2015. For beef cattle, a continuous reduction of the median was seen from 0.2 in 2011–1 over 0.1 between the second half-year of 2011 and 2013 to zero from the first half-year of 2014 until the end of the observation (see **Figure 1**). The proportion of holdings without antibiotic usage increased during the whole observed time period in all three production types. The most obvious change occurred in beef cattle holdings; here, the proportion of holdings without antibiotic usage increased from 22.2% in the first half-year of 2011 to more than half of the participating beef cattle holdings (54.5%) in 2015–2. In dairy cows, the proportion increased from 1.3 to 11.2%, and in dairy calves from 16.1 to 25.1%. Regarding the production types dairy cow and dairy calf, a trend test over the first six half-years showed an increasing linear trend (dairy cows: $p = 0.017$; calves: $p = 0.004$). If the time-period was extended to all eight half-years under study, this turned into a quadratic effect (dairy cows: $p = 0.006$; calves: $p < 0.001$). For beef cattle, this model approach was not feasible due to a large extend of zero antibiotic usage in this production type. Therefore, zero inflated data was observed and no computable results were reported here.

Regression Models

For dairy cows and dairy calves, linear regression models with different transformations were applied to assess the impact of several factors on the farm specific semi-annual TF. The best results for dairy cows were achieved using the square root transformation. **Table 2** shows the effects of each variable, the mean TF in the corresponding category, as well as the associated 95% confidence intervals. The calculations show that only the general factor "time" had a statistically significant impact on the TF in dairy cows. The random factor "veterinarian" had a statistically significant impact on the TF in dairy cows ($p < 0.001$).

For dairy calves, the best fit of the model was observed using the logarithm transformation after adding 0.1. In addition to the factor "time," the factor "farm size" had a statistically significant effect on TF in dairy calves (see **Table 3**), and the average estimator increased with increasing farm size. Between farms in the middle and lower thirds of farm size, an increase of the mean estimator from 0.46 to 0.70 and a clear shift of the confidence interval was evident. The factor "veterinarian" had a statistically significant impact on TF in dairy calves ($p < 0.001$). The distributions of the residuals of multi-factorial models with different transformations for the TF for dairy cows and dairy calves are available as **Supplementary Data**.

In beef cattle, a logistic regression model was adapted. **Table 4** shows the results for beef cattle farms with antibiotic use in general (yes vs. no) and estimated odds ratios with associated 95% confidence intervals. Results show that "time" and "farm size" had a statistically significant impact on the AMU in general. The odds ratios decreased until second half of 2014, which suggested a reduction of the odds to use antibiotics in comparison to odds of not using antibiotics. The odds to use antibiotics in farms of the upper third was 2.8-fold higher than in farms of the lower third. No statistically significant impact of the factor "veterinarian" on the TF in beef cattle holdings could be determined ($p = 0.674$).

The factor "region" had no statistically significant impact on the semi-annual TF in none of the three production types. The estimates of fixed effects regression coefficients and random effects covariance parameters are provided in the **Supplementary Material**.

DISCUSSION

Within the longitudinal study VetCAb data from dairy cows, dairy calves, and beef cattle were observed over several years, facilitating an examination of temporal trends in AMU. For

TABLE 1 | Distribution of the treatment frequency per half-year for dairy cows, dairy calves and beef cattle.

Half-year	Number of holdings	Semi-annual treatment frequency						
		Minimum	5%-quantile	25%-quantile	Median	75%-quantile	95%-quantile	Maximum
DAIRY COWS								
2011-1	474	–	0.3	1.1	1.9	3.2	5.7	11.7
2011-2	474	–	0.3	1.2	2.2	3.4	6.0	11.5
2013-1	178	–	0.0	1.1	2.1	3.5	6.3	11.3
2013-2	175	–	0.1	1.2	2.1	3.4	6.0	10.8
2014-1	173	–	–	1.2	2.1	3.3	7.5	12.7
2014-2	170	–	0.3	1.3	2.3	3.6	6.8	12.7
2015-1	177	–	–	1.0	1.9	3.1	6.5	12.1
2015-2	178	–	–	1.1	2.2	3.8	7.7	12.7
DAIRY CALVES								
2011-1	473	–	–	0.1	0.4	2.1	7.6	20.2
2011-2	473	–	–	0.1	0.5	2.5	9.0	20.8
2013-1	177	–	–	0.1	0.6	2.1	9.5	16.7
2013-2	175	–	–	0.0	0.6	3.1	10.1	20.0
2014-1	173	–	–	0.1	0.6	2.7	8.6	22.6
2014-2	171	–	–	0.1	0.8	3.0	11.3	22.9
2015-1	179	–	–	0.0	0.6	1.6	7.6	13.1
2015-2	179	–	–	–	0.3	1.6	6.3	16.4
BEEF CATTLE								
2011-1	45	–	–	0.0	0.2	0.6	1.8	2.7
2011-2	45	–	–	–	0.1	0.2	0.7	5.3
2013-1	76	–	–	–	0.1	0.5	8.7	16.0
2013-2	76	–	–	–	0.1	0.3	6.5	22.4
2014-1	75	–	–	–	–	0.6	13.1	34.7
2014-2	75	–	–	–	–	0.3	6.4	20.5
2015-1	79	–	–	–	–	0.3	4.3	33.0
2015-2	77	–	–	–	–	0.2	1.8	26.6

–, observed zero; 0, zero by rounding.

this purpose, a calculated semi-annual TF for each holding was used, based on data at farm level. The impact of factors like farm size and region on antibiotic usage was investigated via regression models.

The output of the study presented here is based on voluntary participation, which carries the risk of a selection bias. The number of farms enrolled were proportional to the German farm demographics and therefore a larger number of dairy and a lower number of beef cattle farms were included (12). Due to ongoing participant recruitment, there were changes in the population of study participants, which is typical for open cohort studies. In relation to the number of participating dairy holdings, there was a drop from the pilot to the sentinel study. This decline in participants could not be compensated by new recruitments, and this has to be taken into account when interpreting the smaller collective from 2013 on. In contrast, the number of participating beef cattle farms increased during the observational period. This may be due to the fact that in 2014 the legal monitoring of AMU in fattening animals was introduced in Germany (9). In beef cattle farms, the majority of antimicrobial use data are transmitted online from the software of veterinary practices to the governmental monitoring system. Hence beef cattle holdings could use the same data set to participate in the study with little additional efforts.

As data from routine documentation are used, this carries the ("practical") risk of misallocations to the incorrect production type group. Especially for calf rearing production type, designations were not standardized. Generally, distinctions should be made between calves reared for dairy heifer replacements, calves reared for beef production, and calves fattened for veal production (21, 22). The analyzed dataset contains calves reared on dairy farms for later use as dairy cows or beef cattle. This production type has to be differentiated between calves, which are reared and slaughtered for veal production. When interpreting the results, it should be noted that within the group of dairy calves there is an inhomogeneity of later more clearly separable production type groups. However, the risk of misclassification was minimized by taking into account the type of the farm reflected in the production types included in the database, accompanied by regular communication with the farmers. Concerning dairy cows and beef cattle, misallocations to the production type groups were unlikely.

TABLE 2 | Results of the multi-factorial model with square root transformation for the treatment frequency in dairy cows.

Factor	Category	N	Mean	CI_l	CI_u	*F*-value	*p*-value
Half-year	Global					4.348	<0.001
	2011-1	474	1.783	1.371	2.249		
	2011-2	474	1.962	1.529	2.448		
	2013-1	178	2.109	1.629	2.649		
	2013-2	175	2.079	1.603	2.618		
	2014-1	173	2.006	1.534	2.542		
	2014-2	170	2.231	1.728	2.797		
	2015-1	177	1.783	1.327	2.307		
	2015-2	178	1.954	1.451	2.532		
Farm size	Global					1.174	0.324
	Lower third	607	1.856	1.380	2.403		
	Middle third	647	2.076	1.589	2.630		
	Upper third	745	2.028	1.557	2.561		
Region	Global					2.087	0.195
	Middle	490	1.750	1.232	2.358		
	Northwest	1343	2.131	1.541	2.815		
	East	166	2.088	1.236	3.161		

CI_l, CI_u, Lower and upper limit of the 95% confidence interval.

TABLE 3 | Results of the multi-factorial model with logarithm transformation for the treatment frequency in dairy calves.

Factor	Category	N	Mean	CI_l	CI_u	*F*-value	*p*-value
Half-year	Global					3.606	0.003
	2011-1	473	0.442	0.295	0.643		
	2011-2	473	0.500	0.338	0.723		
	2013-1	177	0.550	0.359	0.820		
	2013-2	175	0.601	0.389	0.907		
	2014-1	173	0.648	0.427	0.963		
	2014-2	171	0.661	0.433	0.988		
	2015-1	179	0.443	0.282	0.671		
	2015-2	179	0.385	0.241	0.589		
Farm size	Global					6.375	0.005
	Lower third	672	0.433	0.272	0.665		
	Middle third	592	0.462	0.295	0.698		
	Upper third	736	0.701	0.472	1.022		
Region	Global					1.167	0.365
	Middle	484	0.423	0.248	0.686		
	Northwest	1343	0.547	0.323	0.890		
	East	173	0.609	0.264	1.281		

CI_l, CI_u, Lower and upper limit of the 95% confidence interval.

To measure the usage of antimicrobial agents, which was calculated on the basis of the number of used daily doses (nUDD). This type of calculation was possible because the information needed is maintained in the ADFs by official regulation in Germany. As described before, ADFs provide detailed information on the actual number of animals treated, number of treatment days and the total amount of antimicrobials used (11). To draw conclusions about the correctness of dosages by comparing the UDD with the labeled dose, additional information is necessary e.g., details of the indication and the veterinarian's decision process, which were not included in our data.

As stated by Pinto Ferreira et al. (23), collecting real use data at farm level is at this time the most accurate way to monitor AMU, because only recording the actual use contributes to avoidance of approximations and resulting data distortion (23). Monitoring systems for AMU at farm or prescriber level provide the opportunity to guide individual preventive or corrective management actions (24). The calculation here is in line with the general therapy incidence concept (25), but real nUDD is used instead of nDDD (number of defined daily doses) and implicit body weight under treatment is used instead of standardized body weights (17). Half-yearly information on the number of livestock places of a holding was not available throughout the project. Therefore, the number of livestock places initially recorded was taken as a basis for calculation (15). We anticipate that the resulting bias is negligible, as we know from transnational data, that the average number of cattle per farm has barely changed over the years considered in Germany (26). Between 2013 and 2015, the half-yearly average number of cattle per farm was 80, 80, 82, 82, 84, and 84, respectively (26). The number of livestock locations for dairy calves is assumed as the number of dairy cows per farm and year. However, it should be taken into account that the period each calf spends on a dairy farm differs from farm to farm. Assuming that this inaccuracy is not related to the number of treatments, it would lead, if at all, to a non-differential information bias. We believe that this assumption is justified, considering the conditions in calf rearing in Germany.

Given the differences of national monitoring systems, transnational comparisons are primarily made based on sales data. In the framework of the ESVAC project a 53% decrease in the overall sales of veterinary antimicrobial agents in Germany

TABLE 4 | Results of the multi-factorial logistic regression model for the treatment frequency in beef cattle.

Factor	Category	N	Use %	Odds ratio	CI_l	CI_u	*F*-value	*p*-value
Half-year	Global						6.251	<0.001
	2011-1 (ref.)	45	77.78	1.000				
	2011-2	45	71.11	1.634	0.579	4.609		
	2013-1	77	56.58	0.522	0.253	1.076		
	2013-2	76	52.63	0.446	0.260	0.764		
	2014-1	75	45.33	0.288	0.137	0.607		
	2014-2	75	37.33	0.217	0.109	0.433		
	2015-1	79	49.37	0.402	0.231	0.701		
	2015-2	77	45.46	0.319	0.164	0.621		
Farm size	Global						9.987	<0.001
	Lower third (ref.)	268	38.43	1.000				
	Middle third	112	57.14	0.898	0.513	1.571		
	Upper third	169	70.83	2.814	1.653	4.793		
Region	Global						5.377	0.073
	Northwest (ref.)	366	58.90	1.000				
	Middle	183	38.80	0.461	0.191	1.114		

CI_l, CI_u, Lower and upper limit of the 95% confidence interval.

between 2011 and 2015 was reported (7). Trends in sales data from other European countries, e.g., Denmark, Belgium, and the Netherlands, showed an obvious reduction of AMU, as well (27). Within the ESVAC project, there is a cross-species documentation of the quantities sold, and it is not possible to allocate the amounts of sold quantities to individual animal species, animal age categories or production types (23, 28). Because an exclusive interpretation of quantities sold cannot provide detailed information on the use of antibiotics, projects and studies of several countries are trying to quantify consumption more closely.

AMU in Dairy Cows

Since dairy cows are not included in the official German antibiotic monitoring system (9), ADFs of dairy farms are collected and analyzed only within this study in Germany. Reporting AMU in dairy cows in the QS-system is based on a small voluntary part of the members only. Therefore, no results have been reported so far (29). Our results show the determined half-year TF ranges between 1.9 and 2.2 days with minor deviations. Compared with the TF calculated for different production types in pigs within the VetCAb-study by Hemme et al. (15) for the same time period, the use of antimicrobials in dairy cows appears to be low but varies over time (15). Merle et al. (30) identified a TF of 0.85 days per 100 days within the VetCAb feasibility study for dairy cows (30). Regarding the shorter observation period, this result corresponds to our results; no temporal trends were identified within this study.

Denmark reports the overall consumption in cattle remained constant between 2011 and 2015 (31). It is emphasized that the vast majority of cattle biomass is comprised by dairy cows, which have a low consumption of antimicrobial agents compared to growing animals (31). In addition to the analysis of sales data, the amount of antibiotics is documented via prescription records including information on animal species, age-group and diagnostic grouping (VetStat). In the annual report, the antimicrobial agents sold for cows and bulls is put together, but that comparability is not given here. To reduce treatment of clinical mastitis the Danish Cattle Association introduced the "milk quality campaign" in 2010 (31).

Using prescription records as data source for AMU is standard practice in the Netherlands as well. The Netherlands Veterinary Medicines Institute (SDa) reports AMU in the Netherlands in dairy farms separately from other cattle. Data is presented as overall antibiotic use, use of dry cow antibiotics, use as mastitis injectors as the defined daily dose animal at farm level ($DDDA_F$). In 2012, 2013, 2014, 2015 the annual median $DDDA_F$ was 2.7, 2.8, 2.2, and 2.1, respectively (32). This seems to be on a similar level, although the TF is working with UDDs and therefore these measures were not comparable directly.

Belgium has also achieved a reduction in antibiotics used in the veterinary field in general between 2011 and 2015 (33). In our study as well as in other studies (30, 34–36), it appears that bovine mastitis is by far the most common indication in dairy cows and reason for treatment with antimicrobial agents (37). In line with this, within the considered period, the majority of antibiotic prescriptions in cattle were dedicated to dairy cows. A Swedish study reported that the treatment of dairy cows constitutes the largest proportion of antibiotic drugs in dairy production, as well (38). The present evaluations include treatment of udder diseases and all treatments in the context of dry-cow therapy. An Austrian study evaluated AMU data with respect to udder diseases of 248 dairy farms in Austria within a 1 year period in 2015 and 2016. The determined mean number of Defined Daily Doses for animals (DDD_{vet}) per cow and year was 1.33 (34). In this study population, treatments for udder disease made up 36.4% of all antimicrobial treatments. Considering that within these evaluations dry cow therapy was excluded, these

results are largely consistent with our results. Since it is well-known that antimicrobial substances applied intramammary for dry cow therapy make up a large proportion of the antibiotic consumption in milking cows (39), research with respect on these different treatment options is needed.

AMU in Dairy Calves

Reporting of AMU in dairy calves not reared for veal or beef production is not mandatory in Germany. Therefore, no direct comparisons to the compulsory system are possible. Our study results show that the median of the TF of dairy calves increases continuously from 0.4 to 0.8 until the 2nd half of the year 2014 and decreases to 0.3 within the year 2015. Antimicrobial agents sold (kg active compound) in Denmark for calves increased between 2012 and 2015. However, except for the age (<12 month), the group of calves is not further determined (31). The reported median of antibiotic use in $DDDA_F$ reported by the Dutch Veterinary Medicine Authority in calf rearing farms in the Netherlands since 2013 is zero (40). Disparities with our results in that case can be explained by different national definitions of the production groups on the basis of gender and age. Due to differing definitions within this production type group, direct comparisons in relation to AMU are not feasible. Consistent with our results, a Swedish study mentioned before that, compared to the treatment of dairy cows, overall drug use for dairy calves is at a low level (38) and used to treat mainly respiratory and digestive diseases if necessary, antimicrobials may be administered in calves orally or by injection (35, 37). Though factors like transport and stress contribute to an increased risk of infectious diseases and become an important determinant of antimicrobial use (41).

AMU in Beef Cattle

In our study over the course of time, the majority of participating farms reduced their use of antibiotics calculated as TF to zero. Compared to the TF of dairy cows, the median of the semi-annual TF was at a very low level already at the beginning of the study and decreased further from 2014 onwards. At the end of the period considered, more than a half (54.5%) of the participating farms did not use any antibiotics at all. Our results are in line with the nationwide monitoring of antibiotic use in beef cattle: the median and the third quartile of TF are zero (42–44). The QS-system for beef cattle shows similar results, too (QS). Therefore, it can be assumed that in the present collective a serious selection bias is unlikely. The reported median of antibiotic use in $DDDA_F$ reported by the Netherlands Veterinary Medicines Institute in beef farms is zero since 2013, as well (40).

Factors Associated With AMU

Several studies have already examined associations between factors such as farm size, region, disease incidence and antibiotic usage in cattle (45–48). To put these factors in relation with the AMU data of cattle within the VetCAb collective, regression models have been calculated for each production type. Hence, mapping the effects of farm size, region and the veterinarian in a temporal context is facilitated.

In dairy cows, the estimated means of the TF rose with the increasing farm size in this study. However, the results of the model also demonstrate that there is no statistically significant impact of farm size on TF. Gonzaley Pereyra et al. (45) observed no significant association between herd size and antimicrobial use in dairy cows from 18 milking herds, as well (45). In contrast, an increase in subclinical mastitis with increasing numbers of cows on Swiss dairy farms was found by Doherr et al. (49). Hill et al. studied dependencies between herd size and antimicrobial treatments of diseases like mastitis and lameness on dairy operations in the United States and found that with increasing herd size, herd-level disease prevalence increased. However, with increasing herd size within-herd prevalence seemed to decrease (46).

In calves, the estimated means of the TF rose with increasing farm size in this study, showing a statistically significant impact of farm size on the TF. These results are in line with the results of other studies: the purchase of calves from dairy farms is common and known to be one of the biggest risk factors for disease in dairy calves (50). Most of the indications for antibiotic treatment in calf production are linked with respiratory disease and enteritis (37). Frequency of respiratory tract infections have also been linked with larger calf group sizes (51). Here a direct comparison is not given, since the group size in which the calves are held was investigated and not the total number of livestock locations. Summarizing this, our findings on the impact of farm size on the frequency of antibiotic treatments seem plausible due to usual management practices in calf rearing.

For beef cattle, a very small number of antibiotic treatments were documented within the considered time period. Consequently, a logistic regression model was calculated, to estimate the overall chance of AMU in relation to a given reference. However, the number of animals treated, the duration of treatment and the frequency of application are not included in the model. Results show a statistically significant impact of farm size on the AMU in beef cattle. Beef cattle are kept in groups and the purchase of calves from several stocks is common (41). It can be assumed that consequently in larger beef herds the possibility for pathogenic exchange and the risk of infectious diseases increases. To the best of the authors' knowledge, research on the impact of farm size on antimicrobial treatments in beef cattle is limited and further studies are needed.

Taking into account structural differences in terms of livestock density and forms of animal husbandry, a regionalization of Germany into agriculturally structurally typically regions was carried out (20). Although it is assumed that a region may be a surrogate for management-related differences due to environment, geography, weather and resources availability that might affect AMU (52, 53), our study showed that in all three analyzed production groups the factor region has no statistically significant impact on the TF.

Pursuant to the current model calculations, the veterinarian has a statistically significant impact on the TF in dairy cows and dairy calves. Possible reasons could be different specializations and experiences of the veterinarians, related to individual prescription behavior influenced by multiple factors

like different treatment durations and selection of drugs (54). Regarding prescribing behavior, Speksnijder et al. found that an increasing experience of the veterinarian is associated with being less concerned about possible veterinary contributions to AMR and also being less concerned to prescribe antibiotics to prevent animal diseases (55). In another study, Gibbons et al. determined by means of a questionnaire the factors influencing the choice of the antimicrobial prescribed. It emerged that the majority of surveyed veterinarians (95.7%) considered that the choice of antimicrobial prescribed "often" or "always" was influenced by the veterinary's prior experience of using a drug for a specific condition (56). However, Cattaneo et al. found a negative relationship between years of practical experience and knowledge about consequences of AMR in bovine veterinarians (57). Our results of the logistic regression model show that the veterinarian has no statistically significant impact on the general AMU in beef cattle. This can be explained by the fundamentally different approaches of the two regression models: in context of the treatment of infectious diseases pursuant "good veterinary practice" (58) and corresponding guidelines (59, 60), veterinarians may differ supposedly more in terms of dosage and duration of antimicrobial treatment, as in terms of whether an antimicrobial drug should be applied. Nevertheless, it should be noted that this factor is not adequately investigated in this study and was only modeled as a variable effect within the regression models.

In general, results demonstrate large differences in antimicrobial usage patterns between the production types in bovine livestock. The fact that the compared studies come up with different findings is likely due to the attributes of the particular study population in terms of age groups and production sectors used in each study. Production type specific antibiotic usage data is providing the basis for risk assessment and the recommendation of appropriate countermeasures for prevention of AMR. The results of the present survey emphasize the need for monitoring and evaluating each cattle production sector separately, considering the respective characteristics (61).

CONCLUSIONS

According to our study results, antimicrobial use in dairy cow and dairy calf holdings in Germany varied on a low level across the period observed. In beef cattle holdings a reduction in antimicrobial usage was evaluated. To enable comparisons of the magnitude of antibiotic consumption across regions or countries, production groups should be defined more clearly. Furthermore, as "farm size" has a statistically significant impact on the magnitude of consumption of antibiotics, this should be regularly considered over time. To achieve the overall objective, the reduction of antimicrobial usage and antimicrobial resistance, science based actions need to be taken, reviewed, and adjusted if necessary taking into account the accompanying variables. Regular adaptation of monitoring and benchmark systems is a crucial element in this effort.

DATA AVAILABILITY

The data were collected on an individual basis from farmers and veterinary practitioners. Each participant gave written consent with the understanding that data would not be transferred to a third party. Therefore, any data transfer to interested persons is not allowed without an additional formal contract. Data are available to qualified researchers who sign a contract with the University of Veterinary Medicine Hannover. This contract will include guarantees to the obligation to maintain data confidentiality in accordance with the provisions of the German data protection law. Currently, there exists no data access committee or another body who could be contacted for the data. But for this purpose, a committee will be founded. This future committee will consist of the authors as well as members of the University of Veterinary Medicine Hannover and members of the funding institution (Federal Institute for Risk Assessment). Interested cooperative partners, who are able to sign a contract as described above, may contact: LK, lothar.kreienbrock@tiho-hannover.de.

ETHICS STATEMENT

Data used within this study is based on mandatory application and delivery forms (ADF), which was provided voluntarily by farmers and veterinarians, signing individual written consent data to be used by the study team only. Our research does not involve any regulated animals and there were no scientific procedures performed on animals of any kind. For this reasons a formal approval by an ethical committee was not necessary under the provisions of the German regulations.

AUTHOR CONTRIBUTIONS

KH and LK: conceptualization, formal analysis, investigation, and writing—original draft. KH and MH: data curation and validation. LK: funding acquisition and supervision. KH, IR, MH, and LK: methodology. KH: project administration. MH: software. KH, NW, AK, and LK: writing—review and editing.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2019.00244/full#supplementary-material

Image S1 | Distribution of the residuals of the multi-factorial model with logarithm transformation after adding 0.1 for the treatment frequency in dairy cows.

Image S2 | Distribution of the residuals of the multi-factorial model with logarithm transformation after adding 1 for the treatment frequency in dairy cows.

Image S3 | Distribution of the residuals of the multi-factorial model with square root transformation for the treatment frequency in dairy cows.

Image S4 | Distribution of the residuals of the multi-factorial model with logarithm transformation after adding 0.1 for the treatment frequency in dairy calves.

Image S5 | Distribution of the residuals of the multi-factorial model with logarithm transformation after adding 1 for the treatment frequency in dairy calves.

Image S6 | Distribution of the residuals of the multi-factorial model with square root transformation for the treatment frequency in dairy calves.

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ORIGINAL RESEARCH
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Exploring Perspectives on Antimicrobial Use in Livestock: A Mixed-Methods Study of UK Pig Farmers

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Increasing levels of antimicrobial resistance in human and veterinary medicine have raised concerns over the irresponsible use of antimicrobials. The role of administering antimicrobials in food producing animals most frequently falls to the farmer, therefore it is essential that their use of antimicrobials is both optimal and responsible. This study sought in-depth information on the drivers behind antimicrobial use behaviors and farmer attitudes to responsible use using a mixed-methodological approach. Initially, in-depth semi-structured interviews were conducted with a purposively selected sample of farmers ($n = 22$). A thematic analysis approach was taken to identify key themes from these qualitative data. The generalizability and variation of these themes was then tested on a larger randomly selected sample of pig farmers through a questionnaire study ($n = 261$). The influences behind antimicrobial use were complex with multiple drivers motivating decisions. There was no consensual opinion on what farming systems resulted in either a low or high antimicrobial requirement however, farmers reported that good management practices, low stocking densities, and a high health status were associated with low antimicrobial use. Farmers expressed desire to avoid the long-term use of in-feed antimicrobials, but identified barriers to discontinuing such behaviors, such as pig morbidity, mortality, and economic losses. The high cost of antimicrobials was described as a motivation toward seeking alternative methods of controlling disease to prophylactic use; however, this expense was balanced against the losses from an increased burden of disease. The high financial costs involved in pig production alongside the economic uncertainty of production and pressure from retailers, were identified as limiting the scope for improvements in pig accommodation and facilities which could reduce the antimicrobial requirements on farm. Long-term, sustainable and economically stable relationships between retailers and farmers may allow farmers to make necessary investments in improving management and housing in order to reduce antimicrobial use. Greater use and more widespread deployment of effective vaccinations were highlighted by farmers as being a feasible alternative to antimicrobial use in preventing disease.

Keywords: antimicrobial resistance, antimicrobial, antibiotic, antimicrobial use, farm animal, behavior, prescribing, mixed-methods

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INTRODUCTION

There is increasing concern over the threat of antimicrobial resistance to human and animal health, with growing efforts by medical and veterinary professions to minimize prescribing and ensure that use is justifiable (1, 2). Antimicrobial use in livestock raises concerns over the potential public health implications from the transfer of resistant bacteria from animals to humans (3–5). A UK government commissioned review on antimicrobial resistance, led by Lord O'Neill, placed the livestock sectors under increasing pressure to collect baseline antimicrobial use data and to set species specific reduction targets (4). In the UK, the pig industry was found to have the highest antimicrobial use across the species sectors in 2015, with a baseline figure of 263.5 mg/PCU compared with the national cross-species figure of 57 mg/PCU (6, 7).

Antimicrobial use in pigs has been under the spotlight with the formation of working groups and research initiatives striving to address this high use and to promote responsible practices (8–10). Practices commonly employed in the pig sector such as the use of antimicrobials for disease prophylaxis and the commonality of the administration of in-feed antimicrobials (11–13) have been associated with high and indiscriminate antimicrobial use (4, 14). These behaviors coupled with the relatively high sales of antimicrobial products, authorized for use solely in pigs, have highlighted pigs as a priority species in the UK and Europe for gaining a better understanding of prescribing and use (15, 16).

There are diverse opinions held by both farmers and veterinary surgeons as to what behaviors are considered to be responsible and what routes should be taken to reduce indiscriminate antimicrobial use (17–20). Typically, most research has focused on the role of the veterinary surgeon in antimicrobial use decisions (21, 22), however, the role of actually administering antimicrobials typically falls to the farmer. Around 92% of UK pig production is overseen by a farm assurance scheme which require, as a minimum, a veterinary visit quarterly (23, 24). Thus, whilst the veterinary surgeon oversees the antimicrobial prescription or supply, and provides advice through a veterinary health plan, there is some freedom of choice with regards to antimicrobial use by UK pig farmers.

In human medicine, antimicrobial practices have been found to be motivated more by drivers relating to the social context of the prescribing environment such as managing time pressures, patient outcomes, relationships with patients, and a physicians' perceived role within the hospital than by concerns over antimicrobial resistance (25–28). Similarly, Bellet reported that drivers relating to the herd productivity, animal health, and welfare motivated anthelmintic use in dairy production; often to the detriment of considerations over anthelmintic resistance (29). Food producing animals occupy a unique position whereby animal management and the economic viability of a farm influence the antimicrobial use decisions of veterinary surgeons and farmers (19, 20, 26). Therefore, there is a need to explore antimicrobial use practices within the context of a pig farm.

The voluntary approach taken to antimicrobial use reduction in the UK coupled with the unique animal husbandry and management systems employed, place the UK pig sector in a unique position in comparison to other European countries. For example, around 40% of the UK breeding herds are raised on outdoor units, a feature which is particular to the UK, and is accompanied by diverse challenges compared to indoor breeding systems (30, 31). It is therefore essential that these and other drivers are explored further with farmers due to their direct effect on antimicrobial use on farms.

This study used a mixed-methods approach to describe pig farmers' antimicrobial use behaviors and explored attitudes to use in pig production in the UK. Thus, not only did the study describe what farmers reported to practically do, with regards to antimicrobial administration, but it also explored attitudes and perceptions to antimicrobial use behaviors. Consequently, the study was able to identify any mismatch between "desirable" behaviors, those are behaviors described in the guidelines as promoting prudent antimicrobial use, and "actual" behaviors reported by farmers. For example, an aspiration by the farmer to reduce antimicrobial use on the farm but barriers beyond their control limiting the scope to do so. The study builds on previous work which exploring veterinary surgeon perspectives to antimicrobial prescribing in the UK pig sector and focuses on the farmer as the end user (18, 22). At present, there are no published studies, which explore in-depth farmer perspectives on antimicrobial use in UK pig production, and as such, the study addresses a current knowledge gap. It is of particular importance due to the unique approach to both antimicrobial use policy and pig production taken in the UK.

METHODS

This study used a mixed-methods approach to explore UK pig farmers' perceptions on the balance between the costs and benefits of antimicrobial use in pigs. Individual semi-structured qualitative interviews allowed a more detailed exploration of farmer attitudes and perceptions around antimicrobial resistance and use; participants were free to discuss potentially emotive subjects on a one-to-one basis without the influence of other farmers (32). Subsequently, the themes were explored on a representative population of UK pig farmers to clarify themes, identify variation in the wider attitudes with regards to antimicrobial use in UK pig production.

Participant Sample Population

The sample population was identified from the Department for the Environment and Rural Affairs (DEFRA) June Survey of Agriculture and Horticulture, 2011. A stratified random sampling methodology was employed to select participants based upon the type of farm, the number of sows/pigs on holdings. This sample was then further stratified by farm size with sampling proportional to the total number of pigs represented by that farm size group; such that large farms which represent the majority of pig numbers were not underrepresented (33) (**Table S1**). For the qualitative sample 150 farms were identified in England based on this sampling frame and farmers were invited to opt out from their telephone numbers being made available to the study. Existing contacts in the pig industry were used to sample

farmers from Scotland however, Wales was excluded from the qualitative study due to the small number of commercial pig units. A purposive sampling approach was adopted to identify 22 participants as it enabled the identification and selection of information-rich participants for the qualitative interviews (34). Farmers were identified from a wide spectrum of farm types to ensure that the qualitative data encompassed knowledge and experience from across the pig sector (35).

For the questionnaire sample, 1,500 farms across England (92% of sample), Wales (<1% of sample), and Scotland (7% of sample) were selected using the aforementioned methodology (**Tables S1, S2**) to reflect the regional breakdown of the breeding herd (33). In order to avoid repetition, the questionnaire sample was distinct from the farms selected for the qualitative sample.

Qualitative Methodology

Qualitative in-depth face-to-face semi-structured interviews were conducted with the pig farmers. An interview guide was designed based on a review of the literature, current issues surrounding antimicrobial resistance and results from focus groups previously conducted on the drivers of antimicrobial use in pigs (36) (**Figure S1**). The interview guide was constructed based on Lofland and Lofland's guide to preparing a qualitative interview (37). The interview guide provided key topic areas which were used to prompt and encourage farmers to express their views however, free conversation was actively encouraged. Open questions were used to encourage farmers to express their views around antimicrobial use in pigs. Interviews were undertaken by the author (LC) with an additional author (SL) also present for a number of interviews.

The interview audio recordings were transcribed verbatim, anonymized and the transcripts were transferred into Atlas.ti V.7.7.1 (Atlas.to Scientific Software Development) for data management. A theoretical approach to thematic analysis was used in which the coding of the transcripts were guided by the authors' pre-existing coding frame from an earlier focus group study (36). To ensure consistency in the analysis technique the approach described by Braun and Clark was adopted where the transcripts were read iteratively and coded data fragments were reviewed and classified to form minor themes (38). These minor themes were then further refined into major themes based on common subject areas. Themes were evaluated by a multi-disciplinary team to ensure that each was distinct, meaningful, and relevant to the research question (34). It was concluded that data saturation had been achieved when no new themes were defined from the interview transcripts and after that no further interviews were conducted.

Questionnaire Methodology

The questionnaire content was based on the results from the qualitative interviews and consisted of the following four sections:

- Farm and participant information;
- Current opinion on antimicrobial use in pigs;
- Pig diseases and antimicrobial use on farm;
- Responsible antimicrobial use.

Open and closed questions were used with Likert scales to gauge opinion on agreement or importance. The questionnaire was created in Microsoft Word for postal distribution on 5 January 2015. A reminder postcard was sent to non-responders 3 weeks later and a second copy of the questionnaire was sent a further 3 weeks later to non-respondents.

Data were analyzed using Microsoft Excel 2010 (Microsoft Corporation, Redmond, Washington, USA) and SPSS Statistics 22.0 (IBM SPSS Statistics for Windows Version 22.0. Armonk, NY: IBM Corp). Descriptive statistics relating to the demographic information of respondents and their respective farms were produced and percentages determined for categorical response questions. Open questions were analyzed using a thematic approach. The open question responses were transferred into Atlas.ti V.7.7.1. (ATLAS.ti Scientific Software Development) for analysis. The free texts were re-read and the ideas generated were categorized and linked to form distinct codes. These codes described the thematic content of the data.

The study sought to explore the risk factors for antimicrobial use in the context of specific disease syndromes in pigs. Therefore, logistic regression analyses were used to determine drivers associated with antimicrobial treatment for specific disease syndromes on the respondents' farms in the year preceding the questionnaire study. Exploratory variables related to the pig density of the farm location, the housing and feeding characteristics of the farm, pig husbandry systems employed, the vaccination status of the herd, and the number of sows or pigs on the farm were assessed. Variable selection was based on risk factors for key disease syndromes identified by participants in the qualitative enquiry of this mixed-methods study.

Variables were assessed for each outcome using a Likelihood ratio (for categorical variables) or univariable logistic regression (for continuous variables) and any variables with $P < 0.25$ were tested for inclusion in multivariable models. The continuous variables (number of sows or pigs) were not normally distributed and were log-transformed in a natural log base 2 to compensate for the skewedness of these data. Therefore, the odds ratios were associated with a two-fold increase in the predicted variable. Models were built manually using a step-wise backwards elimination approach; the variable with the highest *P*-value was removed at each step. Two-way interactions of significant main effects were also tested. Variables were retained if their exclusion resulted in a likelihood ratio test statistic of $P < 0.05$ or if there was evidence of confounding.

Ethical Approval

Ethical approval was granted from the University of Liverpool Veterinary Science Research Ethics Committee and the DEFRA survey control unit prior to commencing the study interviews.

RESULTS

Interview Participants

A total of 22 interviews were completed with farmers from England and Scotland between April 2013 and March 2014. In the sample of 150 English farms, 30% of the farmers contacted over the telephone chose to opt out of the study. Forty-three

participants from the remaining farmers were invited to take part in the study and 21 declined; reasons given included low staffing levels, a lack of time and harvest time. Therefore, 20 interviews were arranged within England using the database and a further two interviews were conducted in Scotland using existing contacts. Both of the farmers contacted in Scotland agreed to take part in the study. Interviews lasted between 30 and 90 min with an average length of 45 min. Demographic information on the farmers included in the questionnaire study are described in **Table S3**.

Questionnaire Respondents

In total 511 (35%) participants responded however, only 261 of these were completed questionnaires (useable response rate was 18.1%); 250 were returned not completed or the questionnaire was returned to the researcher as the address was incorrect. The main reasons stated for non-response were farmers no longer keeping pigs (62%) or a duplicate listing of the same farm under two addresses (21.2%).

The majority of respondents were managers of a single unit (56%, $n = 261$) or multiple units (14%, $n = 261$), whilst 14% ($n = 261$) were independent farm owners. The majority of respondent farms (50%, $n = 261$) had only one member of staff, with 42% ($n = 261$) having two staff members and 8% ($n = 261$) having three or more. Farm managers oversee either independent farms or contract farms. The latter are owned and managed by a larger agribusiness (39). The questionnaire did not capture information on whether respondent farms were either independent or contract farms nor any information on the relationship of the farm with retailers or industry. There was wide variation in the number of pigs on farms with a median of 155 breeding sows and 1,150 feeding pigs on farms (**Figure S1**). The majority of the respondents worked on indoor units and only a small proportion of farms were classified as specialist with 4.6% ($n = 259$) of respondents being from organic farms and 1.9% ($n = 259$) being from specialist breeding units (**Table S4**).

Overarching Themes

The study results revealed three major themes that influenced farmer attitudes with regards to antimicrobial use practices; farming systems; farm management strategies; and farm-level economics. These themes revealed a complex relationship between the farming system, quality of the farm management, and the antimicrobial requirements of the system. These major themes were not discrete and there was overlap between minor themes within them. For example, the economics involved in different farming systems is presented under the major theme of farming systems but is also an important contributor to the major theme of farm-level economics.

Farming Systems

Farming systems were the most commonly discussed major theme across the qualitative interviews. This included all features relating to the farming system adopted such as husbandry practices, farm facilities, and biosecurity measures. Farmers frequently identified that farming systems had a major influence on the total amount of antimicrobial required on a farm. Additionally, farming systems were found to be related and linked with all of the major themes reported from the interview transcripts.

Farmers expressed strong but diverse opinions as to how farming, management and housing systems related to the health and welfare of pigs and consequently antimicrobial use. There was disagreement on what farming system participants' considered to be advantageous for the health of pigs; indoor or outdoor housing; slatted or straw-based pig accommodation. The majority noted that there were limitations and advantages to all production systems and that such contrasts were likely to result in a diverse range of disease conditions; with specific bacteria and viruses prevailing in some systems and being absent from others.

> *"I think every system's got its strengths and its weaknesses, and every system exposes or isolates an animal from certain bacteria or virus[es]..."* (F004)

Unsurprisingly there was a tendency for participants to express more detailed opinions on farming systems that they were more familiar with. For example producers with experience of outdoor production predominantly considered that outdoor breeding herds were likely to have lower antimicrobial use when compared to herds housed indoors and often described the outdoor environment as a more natural system for the sow, which had a positive effect on their health and welfare.

> *"I would say outdoor breeding is certainly a very, very low user of antibiotics… outdoors is a very natural system. The animal takes care of itself…"* (F018)

In contrast, a perception held solely by indoor producers expressed that sows and piglets on outdoor units may suffer negative health and welfare implications due to the extreme temperatures experienced.

> *"… an outdoor pig, is not very happy in February. It's not covered by fur or feather. And it's not very happy in the summer when it's 80 degrees..."* (F005)
> *"When you look at the weather we've had the last two winters, pigs have frozen to death outside in farrowing huts and drowned in farrowing huts."* (F006)

An association between the farming system and the economics of production was identified by farmers. For example, whilst outdoor production was perceived by some as beneficial in minimizing antimicrobial requirements, farmers noted that the scope for outdoor production was limited as it was deemed less economically efficient. For example, outdoor was identified as producing fewer pigs per sow when compared with indoor systems. Additionally, participants expressed the opinion that intensive agriculture was necessary in order to produce enough meat to satisfy consumer demand.

> *"I started off with outdoor pigs, and it works well, but you can't produce the number of pigs from an outdoor system as you can from a well-run indoor system."* (F022)

"The outdoor bred British pig isn't going to feed the world; in all honesty… it will be from intensive people." (F007)

The housing of feeding pigs on slatted floor systems sparked two opposing views amongst participants; some participants considered slats to be advantageous for pig health as they separated the animal from feces and urine. Conversely, the concept of the pig being housed above a slurry pit was not viewed to be a healthy environment for the pigs. In the following two examples participants used examples from human public health to justify their contrasting opinion.

"The Victorians were the ones who back in the 19th century separated the humans from muck, and brought sanitation, and that saw a huge reduction in disease… It's healthy for the pigs. It's more economical. One of the reasons it's more economical is the fact that we have to use less medicines, any in-feed/water, whatever." (F001)
"The worst thing a pig does is get stuck in a confined area, with a fan environment, the standard way. They are sitting above a sewer. They sit on the slats above sewerage. Well yes, that's a very healthy way to live isn't it? Look at the trouble we had in London, in the early part of the century, with the Black Death and the plague and all the rest of it." (F008)

Low stocking densities and maintaining a high health status were associated with a low disease burden and minimal antimicrobial use by farmers. However, a minority of participants expressed concerns that a high health status herd may be vulnerable to novel disease due to an inherent immunological naivety to new pathogens.

"If you want to reduce the drug usage in any livestock sector, reduce the stocking density, whether it is indoors or outdoors." (F010)
"I think if you keep the health status up… it does cut your use of antibiotics markedly." (F014)
"Health status… there is the potential… that everything is then that clean that you have had no pressure to a bug, and when something does come around, it knocks everything sideways." (F020)

Questionnaire respondents were asked their opinion on which management systems have the highest and lowest use of antimicrobials (**Figure 1**). The majority of respondents identified that high health status pig herds, systems sourcing pigs from a single source, well-managed units, and an all-in-all-out pig flow system were features associated with low antimicrobial use. Conversely, systems sourcing pigs from multiple sources, a continuous pig flow system and a high stocking density were linked with a high antimicrobial requirement. There was a spectrum of opinions with regards to whether outdoor or indoor systems have higher antimicrobial requirements although the majority of respondents shared the view that outdoor farrowing systems had a lower antimicrobial requirement when compared with indoor farrowing. In parallel with the qualitative results, opinion was divided between whether slatted or straw-based flooring systems were advantageous for minimizing antimicrobial requirements for pigs.

Exploration of farmer attitudes to management initiatives that would potentially drive a reduction in the total amount of antimicrobials used in the UK pig industry were explored in the questionnaire (**Table 1**). The results showed that there was widespread agreement amongst respondents that eradicating swine dysentery, modernizing pig accommodation, more effective and a wider range of vaccinations would be beneficial in reducing the total amount of antimicrobials used in pigs. Conversely, in parallel with the qualitative study, questionnaire respondents identified that the poor availability of highly skilled stock-people was a barrier to reducing total antimicrobial use in pigs.

Farm Management Strategies

The association between farming systems and antimicrobial use on farm was explored in greater detail through the questionnaire study. Firstly, farmers were asked about antimicrobial use on their farm in the year preceding the questionnaire and the conditions that antimicrobials were used for in different age categories of pigs. Lameness was reported to be the most common disease requiring antimicrobial treatment in both farrowing sows and dry sows, whilst gastrointestinal disease was most commonly reported in piglets and respiratory disease in feeding pigs (**Table 2**).

Logistic regression analysis was used to explore the association between management features and whether a farm had used antimicrobials in the year preceding the questionnaire in each age category of pig; the final multivariable models are shown in **Table 3** and univariable tables in **Table S5**. The farrowing sow and dry sow groups were more likely to have required antimicrobial treatment for lameness if they were housed on a farm with a greater number of sows. Conversely, dry sows were less likely to have required treatment with antimicrobials for lameness if they were housed in a closed herd in comparison to an open herd.

Feeding pigs were found to be more likely to have required antimicrobial treatment for respiratory disease if they were housed on a farm with a greater number of feeding pigs and if they were on farms with a vaccination programme for Enzootic Pneumonia. However, it is worth noting that only 10% ($n = 261$) of farms vaccination their feeding pigs against Enzootic Pneumonia. Feeding pigs were at a lower risk of having required antimicrobial treatment for respiratory disease if they were housed on a closed farm. Piglets housed on indoor farms on slatted or straw-based flooring were at a greater risk of requiring antimicrobial treatment for gastrointestinal disease when compared with piglets on outdoor units. In addition, piglets on farms with a vaccination programme for Porcine Reproductive and Respiratory Syndrome virus (PRRSv) in their sows were more likely to have required treatment for gastrointestinal disease compared to those from farms which do not have a vaccination programme in place. Only 18% ($n = 261$) of farms vaccinated their sows against PRRSv.

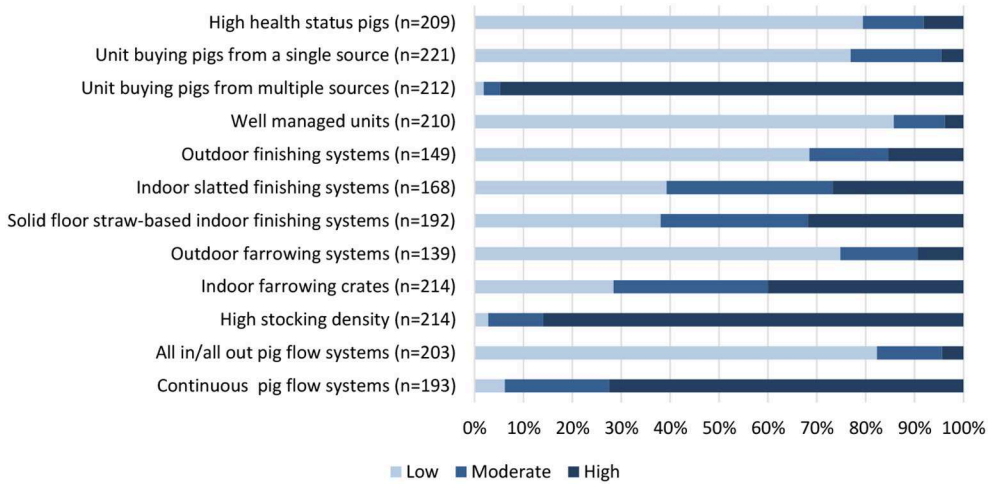

FIGURE 1 | Questionnaire respondent opinion on the antimicrobial use requirements of different management features; low, moderate, or high.

TABLE 1 | Questionnaire respondent attitudes to the role of management and economic drivers in reducing the total amount of antimicrobials used in the UK pig industry.

	Barrier	Neutral	Beneficial
MANAGEMENT DRIVERS			
Eradicating swine dysentery from the UK	11.0% (23)	8.0% (17)	81.0% (170)
Modernizing indoor pig accommodation	4.4% (9)	16.5% (34)	79.1% (163)
More effective vaccines	2.8% (6)	6.1% (13)	91.1% (195)
A wider range of vaccines	3.9% (8)	11.2% (23)	84.9% (174)
De-population and re-populating low health status pig herds with higher health status stock	2.5% (5)	18.5% (37)	79.0% (158)
Poor availability of highly skilled stock people	69.9% (137)	16.8% (33)	13.3% (26)
ECONOMIC DRIVERS			
Increased profitability of pig meat prices	4.6% (10)	26.9% (58)	68.5% (148)
Increasing the cost of antimicrobials for farmers	49.2% (103)	40.7% (85)	10.0% (21)
Decreasing the cost of antimicrobials for farmers	19.3% (39)	52.5% (106)	28.2% (57)
Reducing imports from other countries with high antimicrobial use	6.9% (15)	12.0% (26)	81.1% (176)
Prescription obtained from the vet and taken to a pharmacy to get antimicrobials (i.e., no longer sold by vet practices)	61.8% (126)	26.5% (54)	11.8% (24)

TABLE 2 | Frequency of reported disease conditions requiring antimicrobial treatment in different groups of pigs on farms in the year preceding the questionnaire study.

	Gastrointestinal disease	Respiratory disease	Reproductive disease	Lameness
Farrowing sows	7.8% (9)	7.0% (8)	34.8% (40)	50.4% (58)
Piglets	56.5% (95)	17.3% (29)	0.0% (0)	26.2% (44)
Feeding pigs	22.6% (70)	44.1% (137)	0.6% (2)	32.6% (101)
Dry sows	0.0% (0)	7.4% (9)	17.4% (21)	75.2% (91)

Antimicrobials belonging to the penicillin class were the most frequently used across all of the different categories of pigs accounting for 58.9% ($n = 399$) of recorded use. In sows and weaners antimicrobials belonging to the tetracycline class were the second most commonly reported class [13.5% ($n = 104$) and 15.7% ($n = 127$) of all recorded uses, respectively]. Whilst in piglets the fluoroquinolones [17.1 % ($n = 82$) of all recorded uses in piglets] and in finishers, the macrolides [22.1% ($n = 86$) of all recorded uses], were the second most commonly reported class. The overall reported frequency of use of the third and fourth generation cephalosporins was low (1.2%, $n = 399$) and use was only reported in piglets. Similarly, the polymixin group antimicrobial colistin was infrequently reported and only used in the piglet group (2.4%, $n = 399$).

The World Health Organization (WHO) classification for the highest priority critically important antimicrobial (HP-CIA) classes (40), as defined in 2011 (fluoroquinolones, third and fourth generation cephalosporins and macrolides), was discussed with study participants. During qualitative interviews farmers reported awareness of the concerns over the veterinary use of the HP-CIAs and felt strongly that that their use should be responsible.

> *"There are several medicines that are not necessarily banned on-farm, are they, but they're restricted use because of the effect that that has had on human medicine, from what I understand."* (F012)

TABLE 3 | Multivariable logistic regression analysis of respondent characteristics associated with requirements to use antimicrobials for different disease situations in different groups of pigs in the year preceding the questionnaire study.

		No disease	Disease	Odds ratio	Lower 95% CI	Upper 95% CI	LRT *p*-value
LAMENESS IN FARROWING SOWS							
Number of sows on farm (log base 2 transformed)	Median	105	320	1.3	1.1	1.4	<0.001
	Minimum	2	2				
	Maximum	40,000	4,000				
	IQ range	338	624				
LAMENESS IN DRY SOWS							
Number of sows on farm (log base 2 transformed)	Median	40	285	1.5	1.3	1.8	<0.001
	Minimum	2	10				
	Maximum	40,000	7,000				
	IQ range	270	630				
Closed herd	**No**	127 (67.2%)	62 (32.8%)	Ref			
	Yes	41 (58.6%)	29 (41.4%)	0.4	0.2	0.9	0.024
RESPIRATORY DISEASE IN FEEDING PIGS							
Number of pigs (log base 2 transformed)	Median	300	1,990	1.5	1.3	1.7	<0.001
	Minimum	1	16				
	Maximum	73,500	300,000				
	IQ range	1,425	3,114				
Closed herd	No	80 (42.3%)	109 (57.7%)	Ref			
	Yes	42 (60%)	28 (40%)	0.38	0.2	0.8	0.006
Enzootic Pneumonia vaccination status	**No**	119 (50.6%)	116 (49.4%)	Ref			
	Yes	5 (19.2%)	21 (80.8%)	3.1	1.1	9.0	0.037
			21 (60%)				
GASTROINTESTINAL DISEASE IN PIGLETS							
Flooring type	Outdoor	33 (70.2%)	14 (29.8%)	Ref			
	Straw	35 (67.3%)	17 (32.7%)	5.2	2.1	13	
	Slatted	22 (33.3%)	44 (66.7%)	2.1	0.8	5.6	0.001
Sows PRRS vaccination status	No	156 (72.9%)	58 (27.1%)	Ref			
	Yes	10 (21.3%)	37 (78.7%)	5	2	12.1	<0.001

LRT, likelihood ratio p-value.

In contrast, the questionnaire results showed that only 60.2% ($n = 244$) of respondents stated that they were aware of the issue of critically important antimicrobials. Of 122 farmers that attempted to identify critically important antimicrobials from a list of antimicrobials (including common trade names of products used in pigs), <50% of respondents were able to correctly identify HP-CIA products (**Table 4**).

There was general agreement amongst farmers that the quality of the management system was a more important driver in the amount of antimicrobials used than the type of farming system employed; improving management practices was considered to be pivotal in reducing the antimicrobial requirements on a farm.

> *"Any system can be badly managed. Half of the people that keep animals shouldn't be allowed; they should have a license to keep 'bloody' animals. Sorry, I get very cross about it… Management is a huge thing with managing antibiotic use."* (F005).

Farmers suggested that a minority subset of farmers used antimicrobials in some circumstances as a *"management tool"* to compensate for a lack of re-investment in buildings and facilities. In these situations, interviewees felt that there may be improvements in animal husbandry and management systems that could replace the requirement for long-term antimicrobial use, however, these changes may be less economical than the use of medicated feed. This long-term or *"habitual"* use of antimicrobials was commonly cited by participants as an example of irresponsible use. Furthermore, a minority of farmers proposed that an outlying population of irresponsible farmers in some cases use long-term in-feed antimicrobials for their beneficial effects on growth rates in pig herds.

> *"Because some farmers use antibiotics all the way through the finishers… It becomes a habit, I think, to use it. It becomes a crook… management-wise…"* (F011)
> *"Antibiotics has become a prop for poor buildings and bad practice."* (F016)
> *"…a poorly managed farm, the chances are you will use more antibiotics than a well-managed farm. Of course there are always differences, you will get some guy who is very switched on, very well managed, and will use drugs as a growth promoter…"* (F017)

Half of the questionnaire respondents (50%, $n = 118/234$) identified that they had used antimicrobials on their farms for disease prevention and the majority of respondents reported that antimicrobial use for disease prevention and the use of in-feed

TABLE 4 | Antimicrobials identified as HP-CIAs by UK pig farmers ($n = 122$) from a provided list of drugs including both generic and trade names.

Antimicrobial	Number of participants who identified antimicrobial as critical
Amoxycillin (Amoxinsol, Stabox)	45 (36.89%)
Apramycin (Apralan)	12 (9.84%)
Ceftiofur (Excenel, Naxcel)*	30 (24.59%)
Colistin (Coliscour)	6 (4.92%)
Florfenicol (Nuflor Swine)	15 (12.30%)
Fluoroquinolones (Baytril, Marbocyl, Forcyl)*	52 (42.62%)
Lincomycin (Lincocin, Linco-spectin)	18 (14.75%)
Penicillin (Duphapen, Ultrapen LA)	50 (40.98%)
Spectinomycin (Spectam)	5 (4.10%)
Tetracyclines (Terramycin, Engemycin, Aurofac)	32 (26.23%)
Tiamulin (Denagard)	5 (4.10%)
Tilmicosin (Pulmotil)*	4 (3.28%)
Trimethoprim sulfate (Trimediazine, Tribrissen, Norodine 24)	9 (7.38%)
Tulathromycin (Draxxin)*	20 (16.39%)
Tylosin (Tylan)*	22 (18.03%)

* *Shows HP-CIA classes according to the WHO 2012 definition (40).*

TABLE 5 | Questionnaire responses on the justification of antimicrobial use practices in UK pig production.

	Never justified	Rarely justified	Usually justified	Always justified
Antimicrobial use for treatment of pigs with disease	0.4% (1)	2.1% (5)	43.7% (104)	53.8% (128)
Antimicrobial use for disease prevention	18.9% (43)	29.8% (68)	44.3% (101)	7.0% (16)
Antimicrobial use for growth promotion	68.6% (151)	23.6% (52)	7.7% (17)	0.0% (0)
The use of in-feed antimicrobial formulations in pigs	17.6% (37)	25.2% (53)	48.6% (102)	8.6% (18)

formulations was either usually or always justified. In addition, most respondents agreed with the current policy that prohibits the use of antimicrobials for growth promotion, however 22.1% felt that such use was rarely justified whilst 7.2% felt that it was usually justified (**Table 5**).

Both methods identified that the decision over whether or not, and when, to withdraw prophylactic antimicrobials is a problematic one. Interviewees identified a fine balance between the economic cost of disease and antimicrobial costs; with the decision to discontinue medication being a compromise between the two.

> *"…the cost of disease on any commercial unit… is huge. It comes down to what's your attitude in terms of risk and everything else? Sometimes the risk of breakdowns in health is such that… people are really, really reluctant to actually take it [in-feed antimicrobials] out."* (F009)

The questionnaire explored drivers influencing the decision whether or not to withdraw prophylactic antimicrobials using an open question and free text box (responses are shown in **Table 6**). Clinical drivers such as the presence of disease on a farm, mortality rates and efficacy were the most common motivations for the continued use of in-feed antimicrobials whilst non-clinical drivers such as a reduction in herd performance, cost effectiveness, and veterinary advice were less commonly cited. In contrast, the decision to discontinue in-feed antimicrobials was predominantly driven by non-clinical features such as high cost, veterinary advice, and concerns over antimicrobial resistance. Highly skilled staff were identified as an integral part of a well-managed pig unit. Some participants directly linked the quality of staff skills with antimicrobial use. For example, poorly skilled staff were considered to be a limitation in reducing use on some units as stock people who are disinterested and less skilled in their work may use antimicrobials as a short-term solution to a longer term problem.

> *"Good stockmen are worth their weight in gold… If you're not interested and you're not bothered, what's easier than chucking a load of antibiotic food in? It makes it right for the short term, doesn't it?"* (F006)

In contrast, farmers defined good stock people as those with an innate skill and ability to detect any discomfort in the pig herd before it became a major problem.

> *"So the sharper the stock man, the more effective you can deal with issues before it gets out of hand, and make decisions fast in terms of segregation or that sort of thing."* (F013)

The recruitment and retention of highly qualified staff was problematic; they identified that a lack of availability of highly motivated staff was a pressure on the pig industry.

> "*The biggest problem we have as an industry is finding good staff… everybody I talk to is struggling to find people, who want to actually spend time with animals, let alone, are happy to work seven days a week, you know. But that's what animals have to have, a seven day a week commitment."* (F008).

Farm-Level Economics

Farmers focused on farm-level cost effectiveness and profitability when considering the economic drivers behind antimicrobial use decisions. A more detailed discussion of the wider economic aspects of antimicrobial use in pig production, such as food supply chains and the pharmaceutical industry, were beyond the scope of this study and were not concerns volunteered by farmers through the qualitative interviews. The high financial costs involved in pig production, juxtaposed with the economic uncertainty of production, were identified as limiting the scope for improvements in pig accommodation and facilities which could reduce the antimicrobial requirements on farm. Farmers expressed a desire to minimize the economic burden from disease

TABLE 6 | Themes volunteered by questionnaire respondents as influencing the decision to continue or discontinue in-feed antimicrobials on their farm.

Drivers identified by farmers as influencing the decision to continue in-feed antimicrobials on their farm			Drivers identified by farmers as influencing the decision to discontinue in-feed antimicrobials on their farm		
	n	Example quotation		*n*	Example quotation
Known disease issues in pigs	21	*"Had an underlying problem on farm and needed it cleared up"*	High cost	31	*"Can be costly if there are feed spillages and if over treating all pigs instead of injecting 10–20 instead."*
Veterinary advice	21	*"Only if vet considers this wise"*	Improvements in pig health	19	*"The disease burden has reduced"*
To prevent a reduction in herd performance	16	*"Improved performance of pigs"*	Discontinue use when clinical signs no longer present	17	*"If clinical signs have disappeared or receded to us having to confidence to stop feed medication."*
Prevention of disease is better than treating disease once clinical signs are apparent	13	*"Prevention always better. Especially weaning—time of most stress"*	Veterinary advice is to discontinue in-feed antimicrobials	15	*"The vet decides about when to start and stop in feed antibiotics"*
Good efficacy	11	*"We used to because it was easy and effective"*	Ineffective if used long term	9	*"Ineffective if used too often"*
Disease problems occur if in-feed is withdrawn	9	*"Having tried to withdraw antibiotic, disease re-establishes"*	Concerns over antimicrobial resistance	7	*"It helps cause resistance and it is no longer a responsible option to use them long term."*
To maintain a high level of welfare	8	*"Welfare of pig. If stop animal may break down"*	Improvements in weather conditions	6	*"The weather and time of year is a major factor. Pigs can be moved away on another site in summer months so shed can be washed, rested and re-furbed if needed."*
Cost effective to continue with medication	8	*"Prevention has good cost/benefits"*	Personal concern over the ethics of the long-term use of in-feed antimicrobials	5	*"The feeling that things have changed"*
To prevent high mortality rates	6	*"Insurance against unforeseen losses especially if disease is causing no? deaths."*	Industry pressure to discontinue use of in-feed antimicrobials	5	*"Some companies have routinely stopped in feed to impress retailers"*
Respiratory disease problems	5	*"All of the pigs are coughing"*			
Time of year when disease is common	5	*"There are certain times of year that is unwise to stop, you stop in spring when environment is on your side."*			

and associated the absence of disease with low antimicrobial use and thus reduced veterinary costs. This concept echoes the importance placed by farmers on good management for minimizing antimicrobial use.

> *"…you cannot run a pig farm profitably with high levels of endemic disease."* (F009)

Economic pressure was considered by some to limit the scope to reduce antimicrobial use on farm. Whilst many farmers described an aspiration to reduce antimicrobial use, a "desirable behavior," the high cost of re-investing in housing or facilities was identified as a barrier to behavior change.

> *"…accommodation is a key part of improving health, we then need to be able to be reinvesting in quality finishing accommodation. And you need a desire to be able to reinvest the money. So you need some profit to start with."* (F001)

Farmers considered that the high cost of antimicrobials was a motivation toward ensuring that their use was minimal on farms.

> *"…there are huge cost implications with antibiotics… So we're obviously all the while looking to see, "Do we need that in the feed, that antibiotic?" But then equally you look and say, "Well if we don't have it in there, what's the cost of that going to be?... at the end of the day, we're running a business here trying to produce meat for people to eat.""* (F004)

Many identified that such costs acted as an incentive to seek alternative therapeutic and prophylactic methods to antimicrobial use. For example, farmers proposed that the introduction of a vaccination protocol to prevent disease alongside achieving and maintaining a high health status could minimize the costs of antimicrobials and offer farmers greater profitability from the pig herd.

> *"If you can stabilise health and you can manage that health, then, you certainly will be using a lot less reactive-type drugs if you can have good health plans, and have good vaccination programmes with preventative use of those drugs. Then, you should be using less and you should have a more profitable unit, without doubt."* (F022)

Farmers' described that this desire to minimize antimicrobial costs was founded on the substantial economic pressure on the

pig industry to produce pigs at a low cost to the consumer; the majority identified this as a long-term pressure from retailers. Discussion over retailer pressure was emotional and sparked passionate opinions.

> *"At the end of the day we are really pressurising them [stock people] to reduce costs, so we don't want to use medication unless we have to. We would rather do the testing, we would rather use vaccines."* (F018)
> *"Continual supermarket pressure in terms of not paying the right price for the product. Also the feed costs have been ridiculous these last few years… the financial pressure on pig farmers has been extraordinary."* (F013)

The majority of interviewees felt that "decoupling" prescribing and dispensing, such that veterinary surgeons are no longer able to sell antimicrobials, would have little effect on overall antimicrobial use. However, a minority felt that this may be a beneficial intervention to reduce antimicrobial use by some irresponsible veterinary surgeons; who may be driven to prescribe by the ability to profit from antimicrobial sales.

> *"I could see why in the market, there could be an incentive for them to over-prescribe because there was a profit incentive. I would like to think that the vets are responsible enough not to do that, but I could see why, potentially, it could be an issue, and I could see why some countries have split the different services."* (F014)

Questionnaire respondents identified that farm economics and antimicrobial costs could play a role in reducing the total amount of antimicrobials used in the UK pig industry (**Table 1**). There was shared agreement amongst the majority of farmers that increased profitability in pig meat prices and reducing importation from high antimicrobial use countries would be beneficial in reducing the total amount of antimicrobials used in pigs. There was a range of opinions on the effects should the cost of antimicrobials be increased or decreased for farmers, however, the majority felt that it would have little effect on total antimicrobial use in pigs.

The majority of farmers were unsure on the future sustainability of the UK pig industry, an opinion founded on uncertainty over the economic viability of pig production. Whilst farmers considered that pig production had the potential to produce meat at low costs they expressed concerns that the low prices paid by retailers were hurdles to the profitability of pig enterprises. Some participants depicted a cyclical economic landscape in pig production whereby the industry continued going through phases of both growth and decline. However, farmer opinion was divided between those optimistic and those pessimistic as to whether the future would be toward the financial rewards phase of the cycle. Most participants considered that the retailers and associated consumer demands would influence the future and sustainability of the sector.

> *"We are back to the supermarket actually putting their money where their mouth is by continuing to source UK pigs, and because of our regulation, it costs more."* (F020)
> *"I have a mildly optimistic view, mainly because I think the levels it's at the moment are, historically, as low as they've ever been since we developed a pig industry... We've never been self-sufficient in pig meat. I just think the potential's there… Beef and sheep are going to be too expensive. Pig meat can still be produced economically, so I think it has brilliant potential. The rest of Europe eats twice as much as we do."* (F012)
> *"The pig industry, in its cycle, is always moving from – I'd like to say boom to bust, but we don't have much boom, and it's generally bust."* (F001).

DISCUSSION

The study a mixed-methodological approach to identify farmers' perspectives on antimicrobial use behaviors in pig production in the UK and to explore potential routes to antimicrobial use reduction. Farmers described an economic benefit to antimicrobial use in terms of reducing the disease burden on farm, however, this was balanced against the high cost of antimicrobials and a drive amongst farmers to seek alternative methods of preventing disease to antimicrobial use. Farmers held a spectrum of opinions as to the antimicrobial requirements of different management systems; however, there was agreement that good management was key to reducing antimicrobial requirements.

In agreement, the literature highlights that the quality of the management is essential in minimizing the antimicrobial requirements of a farm with farmers describing the importance of an optimal environment for pigs (13, 31, 41, 42). Many identified that a lack of economic certainty had resulted in the inability of many farmers to reinvest in the housing and management improvements needed to reduce their reliance on antimicrobials. Such conflicts are recognized in other studies with Stevens et al. (31) reporting that farmers' who felt that their farm environment could be improved used more in-feed antimicrobials compared to those that did not perceive that improvements were necessary. Similarly, Alarcon et al. (43) highlighted that farmers recognized a need to balance the high cost of disease with augmenting production costs (31, 43).

The adoption of herd management strategies and improved biosecurity may be a more cost-effective and feasible alternative to preventing disease than routine antimicrobial use (44, 45). The most important driver of implementing such measures, or changing behavior, in pig farmers is the potential economic rewards in profitability and reducing antimicrobial costs (19). However, economic uncertainty, fluctuating prices and increasing retailer demands put farmers under increasing financial pressure (46). Farmers cited the unpredictable and downward price trends from retailers as being responsible for the economic instability they had experienced. This has been described as a concern for farmers and veterinary surgeons in the pig sector (43, 47). However, in contrast retailers have been identified as actors in promoting minimal and responsible antimicrobial use behaviors in pig producers (10). Long-term, sustainable, and economically stable relationships between retailers and farmers may allow farmers to make necessary investments in improving management and housing

in order to reduce antimicrobial use. For example, offering economic rewards for low use may incentivize farmers to engage in seeking alternatives to antimicrobials and to optimize use.

It has been proposed that the ability to profit from the sale of antimicrobials may act to incentivize overprescribing in veterinary surgeons (48–50). Whilst the majority of farmers felt that this would not motivate prescribing by most veterinary surgeons a minority, felt that it may drive prescribing by some veterinary surgeons. In agreement, Visschers et al. found that farmers perceived that "decoupling" would have little importance in reducing antimicrobial use in pigs (20) whilst Postma et al. reported that veterinary surgeons felt that retaining the right to sell antimicrobials was a motivation to reducing antimicrobial use (21).

The outcomes from "decoupling" policies are diverse across the countries that have introduced such legislation; ranging from Norway and Sweden, with some of the lowest sales to Italy, with one of the highest (51). The importance of antimicrobial sales for a veterinary practice is highly variable and depends on the relationship of the practice pharmaceutical suppliers and the costing structure of the practice. For example, profit from the sale of antimicrobials often subsidies the costs of veterinary visits for farmers (52). Consequently, any such policy to regulate antimicrobial sales may have wider impacts on the structure of practices, costs of veterinary services for farmers, practice profitability, and on the veterinary surgeon-farmer relationship.

Responsibility for the prudent use of the HP-CIAs in livestock is shared between the veterinary surgeon, as the prescriber and the farmer, as the end user. Thus, there is a need for farmers to be aware of concerns over their use (53) and this is of particular importance with an increasing move from retailers to introduce antimicrobial use policies, which regulate the use of antimicrobials. For example, dairy farmers who are members of the Tesco Sustainable Dairy Group are required to reduce their use of HP-CIAs and to provide antimicrobial susceptibility test results to support any usage on farm (54). Similarly, there has been a move in some countries for retailers to market meat as "raised without antibiotics" in response to growing concerns over antimicrobial use in livestock (55, 56). Therefore, it is important that farmers have an understanding of the HP-CIA classes and concerns about their use in livestock.

Whilst knowledge of the public health concerns over the HP-CIAs are reported as being widespread amongst veterinary surgeons (57), there is no published literature which explores farmer awareness of the issue. All interviewees expressed awareness of the HP-CIAs, however only 60% of questionnaire respondents reported awareness of the issue and less than half of these could correctly identify HP-CIAs from a list of commonly used antimicrobials. This mismatch in participant awareness, despite being drawn from the same sampling frame, may reflect that interviewees consenting to face-to-face interview were more likely to have a pre-existing interest in antimicrobial use and resistance and thus may be more likely to be aware of HP-CIAs. Since this study was conducted there have been numerous education initiatives to raise awareness of antimicrobial resistance and HP-CIA use amongst farmers with the aim of reducing HP-CIA use, alongside overall use (7, 54, 58, 59). In addition, the Pig Veterinary Society published guidelines advising that HP-CIAs should not be used as first line antimicrobial options (60). Thus, the increased communication, from key stakeholders on the importance of prudent use of HP-CIAs has hopefully resulted in greater knowledge on the issue by UK pig farmers since the completion of this study, however this should be reassessed.

The prophylactic use of antimicrobials at group level has been identified as a frequent behavior in European pig production (26, 31, 61, 62), in spite of pressure by the European Parliament to restrict the practice (63). In response there has been a move to evaluate alternative methods of preventing disease (10, 19, 44); a concept desired and favored by farmers in this study. Similarly, other studies have associated the long-term use of in-feed antimicrobials with irresponsible use behaviors (20, 64). However, in parallel with the opinion reported in the literature (11, 18, 26, 31, 36), participants felt that the use of antimicrobials for disease prophylaxis was justified in some circumstances. In contrast, a minority expressed concern that there may be some irresponsible pig producers who use antimicrobials as a long-term "management tool" in place of husbandry improvements.

The decision over whether to continue or withdraw prophylactic medication was problematic for farmers due to the unpredictable nature of disease and the potential costs should disease return on the discontinuation of antimicrobials. These are common concerns amongst pig veterinary surgeons and farmers (47, 64). The importance farmers placed on the cost-effectiveness of these decisions is also shown in a study which identified that economic considerations were crucial in pig farmer decisions on disease control (43). The Pig Veterinary Society advise that the need for prophylactic antimicrobials should be reviewed at quarterly farm assurance visits and this should form the basis for responsible antimicrobial use (65). Further guidance directed at farmers and veterinary surgeons on the importance of reviewing preventive antimicrobials and alternative methods of preventing disease would allow more informed decisions to be made with regards to antimicrobial use for disease prevention.

Highly skilled stock people were perceived by farmers to be an essential component of a well-managed pig unit enabling early disease recognition and prompt antimicrobial treatment. In parallel with the literature farmers reported that not all stock people possess these essential skills (26, 66, 67). Fertner et al. (41) reported that highly skilled staff were better able to identify disease signs early, however, the study reported that veterinary surgeons did not necessarily correlate this with low antimicrobial use (41). This study also highlighted the importance of spending sufficient hours observing pigs in order to recognize any issues in a herd. In other studies the presence of highly-skilled stock people, who show empathy for pigs under their care, has been correlated with positive health, welfare, and productivity parameters in pigs (68–70). Thus, there is a potential for structured education and training for stock people on pig herd health management with a focus on responsible antimicrobial use.

Lameness has been identified as a major driver toward antimicrobial use in sows and is one of the most significant reasons for both euthanasia and early culling in breeding pigs (31, 71). It is of great economic importance to pig production due to its negative effects on sow fertility and herd productivity (72), and the study results identify it as the most important clinical indication for antimicrobial use in sows. Respondents reported sows from herds with a greater number of sows were more likely to require antimicrobial treatment for lameness. The literature reveals contrasting results with some studies reporting that an increase in herd size is associated with a decreased risk for the development of lameness (71, 73) whilst, Willgert et al. notes that factors associated with larger and more productive herds pose an increased risk for lameness in the English pig herd (74). Interpretation of these results need to be considered within the specific context of the study as the findings assess the use of antimicrobials for lameness. Therefore, it may be that stockpersons on larger pig units are more likely to identify and treat lameness, or are more likely to have a proactive prevention plan for lameness and may have better handling facilities to treat lameness when compared with smaller pig herds. Presently there is a knowledge gap with regards to risk factors for lameness in sows which an area which warrants further research.

Respiratory disease was found to be the most important disease syndrome requiring antimicrobials in feeding pigs (31). Conversely, in piglets gastrointestinal disease was more common. Additionally, these were the most frequently reported conditions that required antimicrobial treatment in all groups of pigs across Europe (75). In parallel with the findings for lameness in sows the study results revealed that feeding pigs from herds with a greater number of pigs were more likely to require treatment for respiratory disease. Similarly, the literature identifies that a larger herd size presents a greater risk for respiratory disease when compared with smaller herds (76–78). The policy of maintaining a closed herd, whereby no new animals are introduced, has been associated with improved animal health and productivity as well as lower antimicrobial use (42, 79–81). In agreement, the study revealed that the risk of requiring antimicrobial treatment for lameness in dry sows and respiratory disease in feeding pigs was lower in closed herds when compared to those that were open.

Respondents identified that piglets housed outdoors were less likely to have required antimicrobial treatment for gastrointestinal disease in comparison to piglets housed indoors on a slatted or solid-floor with straw bedding. There are very few studies which explore the relationship between disease status, antimicrobial use and outdoor or indoor production systems, however, Stevens et al. (31) concluded that overall, for all disease conditions, outdoor breeding units spent significantly less on injectable antimicrobials for pigs when compared with indoor breeding (31). A study by Kilbride et al. into pre-weaning piglet mortality found that diarrhea was a more frequent cause of mortality in piglets housed indoors when compared to those reared outdoors (82). However, *Salmonella,* a significant cause of diarrhea in the UK pig herd (83), has been shown to have a higher incidence on outdoor units when compared with indoor farms (84, 85).These findings may reflect that both internal and external biosecurity are easier to implement and maintain on an indoor unit when compared to an outdoor herd (86). In order to fully understand the risks for pre-weaning diarrhea, and the need for antimicrobial treatment in piglets further research into the effects of environment, such housing systems and flooring types is needed. In addition, work to identify and describe effective biosecurity measures to prevent the introduction, or spread, of diarrheal pathogens for indoor and outdoor systems is essential.

Vaccination programmes are used to improve the immunity of pigs, reducing the risk of clinical signs of disease, and consequently reducing the need for antimicrobial treatment. Thus, vaccinations are reported to be an alternative method of controlling disease to antimicrobial use (44, 80, 87). In agreement, questionnaire respondents felt that the availability of more effective vaccinations and a wider range of vaccines would be beneficial in reducing overall antimicrobial use. In addition, interviewees defined vaccination as a feasible alternative route for preventing disease to antimicrobial use and is an area where further research is needed.

The results from the logistic regression analysis contrast with the general principle of a vaccination, as protective against disease, as the study found that having a vaccination programme was associated with an increased use of antimicrobials on farms. This contradiction has been observed in other studies exploring the relationship between antimicrobial use and vaccination in pigs (80, 88). These results may represent the attitudes of farmers or their veterinary surgeons, that using a greater number of vaccinations and antimicrobials is a more effective insurance against disease than using fewer pharmaceutical products (19, 26, 80). Alternatively, these contrasts may reflect that pig herds with vaccination programmes have a higher disease pressure than herds without vaccination programmes and that in vaccinated herds disease is yet to be controlled through vaccination alone. Thus, such farms may be relying on a combination of vaccination and antimicrobials in order to control the clinical signs of disease (80). Furthermore, the results from this study should be considered in relation to the small respondent population that had a vaccination programme in place. Further research to determine the true advantages of vaccination in terms of reducing antimicrobial use is required. This work should include a detailed exploration of the farm-level vaccination programmes including information on the vaccination types used, history of disease pressures encountered on farms and the indications for antimicrobial treatment in pigs.

The adoption of mixed methods acted to combine the strengths of both qualitative and quantitative enquiry to increase the depth and breadth of the understanding of farmers' perceptions on antimicrobial use and how this affected their use behaviors (89). This provided a more complete picture of perceptions and beliefs than either method could have done individually (90–92). For example although interviewees expressed awareness of HP-CIAs, when tested further this was not consistent across the larger population and less than half of questionnaire respondents were able to correctly identify HP-CIA classes from a list of antimicrobial active ingredients.

The study presented an overview of farmers' attitudes to antimicrobial use and as such did not provide a detailed analysis of how respondent demographics may influence antimicrobial use behaviors. With the adoption of a mixed-methods approach, it was beyond the scope of the study to undertake a more detailed statistical analysis of questionnaire responses; such as those seen in purely questionnaire studies into antimicrobial use practices (20, 22, 93). In addition, the questionnaire content focused on findings from the qualitative study and the contrasts between the farming systems, for example, the differences between indoor and outdoor production, opposed to investigating differences between the characteristics of respondent farmers. Therefore, these results provide a baseline of information on farmer attitudes to antimicrobial use in the pig sector as a whole, which warrants further exploration with regards to how respondent characteristics influence antimicrobial use behaviors and attitudes to use.

Although the useable response rate for the questionnaire study was only 18%; overall 35% of the questionnaires were returned, but 62% of those returned were from respondents who were not eligible to be included in the study; most frequently because they no longer kept pigs. The low response rate may have introduced bias as the responders may be different in terms of antimicrobial use and perceptions, to non-responders. Potential reasons for non-response across both the qualitative interviews and questionnaire may be related to the sensitivity of the issue of antimicrobial use in pigs. There has been increasing pressure from the general public, politics, and media regarding antimicrobial use in food producing animals and it is possible that this scrutiny may have resulted in a reluctance for farmers to discuss their current practices for fear of negative consequences (26, 94, 95). In addition, there may be limitations to self-reported behaviors with participants responding to questions in the manner in which they perceive is expected (96, 97), thus, there is a potential in this study that respondents may report antimicrobial use behaviors that they consider are optimal and responsible rather than their actual practices. However, the very open and honest discussion in the qualitative interviews, including discussions on highly emotive subjects such as the potential public health consequences from antimicrobial use in pigs, suggest that the study presents accurate perceptions and behaviors (98).

CONCLUSIONS

Farm profitability and disease burden were reported to be precariously balanced; with farmers identifying that costs and benefits were major drivers in antimicrobial use decisions. Farmers identified that improving management practices and stabilizing prices would be routes through which antimicrobial use can be minimized in the UK pig sector. Further research is needed to identify cost-effective management strategies to reduce antimicrobial use in typical UK production systems.

Antimicrobial use for disease prophylaxis remains an important disease management tool for many producers however, farmers reported a need to seek alternative methods for disease prevention. Providing detailed guidance on reviewing routine preventative antimicrobial use and alternative methods for disease prevention would allow more farmers to make informed decisions on antimicrobial use.

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript and/or the **Supplementary Files**.

ETHICS STATEMENT

Ethical approval was granted from the University of Liverpool Veterinary Science Research Ethics Committee and the DEFRA survey control unit prior to commencing the study interviews.

AUTHOR CONTRIBUTIONS

GP, NW, SL, and SD conceived the study. LC was responsible for drafting the manuscript, undertaking the qualitative interviews along with SL, designing and circulating the questionnaire and analyzing the data. GP, RS, and RP oversaw the questionnaire design. GP assisted with statistical analysis. SL, GP, and ID oversaw the qualitative interview study design and analysis of data. RP and RS provided specialist clinical advice to ensure that the study was clinically relevant. All coauthors played a significant role in the editing and reviewing of this paper.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2019.00257/full#supplementary-material

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Extrapolating Antibiotic Sales to Number of Treated Animals: Treatments in Pigs and Calves in Switzerland, 2011–2015

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To evaluate the contribution of antimicrobial use in human and veterinary medicine to the emergence and spread of resistant bacteria, the use of these substances has to be accurately monitored in each setting. Currently, various initiatives collect sales data of veterinary antimicrobials, thereby providing an overview of quantities on the market. However, sales data collected at the level of wholesalers or marketing authorization holders are of limited use to associate with the prevalence of bacterial resistances at species level. We converted sales data to the number of potential treatments of calves and pigs in Switzerland for the years 2011 to 2015 using animal course doses (ACD). For each authorized product, the number of potential therapies was derived from the sales at wholesaler's level and the ACD in mg per kg. For products registered for use in multiple species, a percentage of the sales was attributed to each authorized species according to their biomass distribution. We estimated a total of 5,914,349 therapies for pigs and 1,407,450 for calves in 2015. Using the number of slaughtered animals for that year as denominator, we calculated a treatment intensity of 2.15 therapies per pig and 5.96 per calf. Between 2011 and 2015, sales of veterinary antimicrobials decreased by 30%. The calculated number of potential therapies decreased by 30% for pigs and 15% for calves. An analysis of treatment intensity at antimicrobial class level showed a decrease of 64% for colistin used in pigs, and of 7% for macrolides used in both pigs and calves. Whereas the use of 3rd and 4th generation cephalosporins in calves decreased by 15.8%, usage of fluoroquinolones increased by 10.8% in the same period. Corresponding values for pigs were −16.4 and +0.7%. This is the first extrapolation of antimicrobial usage at product level for pigs and calves in Switzerland. It shows that calves were more frequently treated than pigs with a decreasing trend for both number of therapies and use of colistin, macrolides and cephalosporins 3rd and 4th generations. Nonetheless, we calculated an increase in the usage of fluoroquinolones. Altogether, this study's outcomes allow for trend analysis and can be used to assess the relationship between antimicrobial use and resistance at the national level.

Keywords: antibiotics, antimicrobial consumption, course dose, pigs, calves

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INTRODUCTION

Use of antimicrobials contributes to the emergence and spread of resistant bacteria in both humans and animals. As early as in the 1960's concerns arose in relation to therapeutic, preventive and growth-promoting treatments in food-producing animals. The fact that most antibiotic classes are administered to treat infections in both humans and animals was one of the major concerns (1, 2). Monitoring antimicrobial usage is therefore a prerequisite to assess the impact of antibiotic treatments on the selection and spread of bacterial resistances. In order to achieve that goal, a number of programs monitoring sales and/or usage of antimicrobials have been established both at national level, for example in Switzerland [ARCH-Vet; (3)], Denmark (4), and international level [ESVAC project of the European Medicines Agency; (5)]. These programs do not only aim at the identification of trends in sales and usage of antimicrobial classes but should also allow establishing a link with changes observed in resistance monitoring programs, thereby providing a basis for risk assessment and evaluation of regulatory interventions (6). In order to assess the association between antimicrobial use and resistance, it is of crucial relevance to obtain consumption data at species or, when possible, production type level; there are several species and production type-specific factors that can impact on the relationship between use and resistance. Those factors include age at treatment, age and weight at slaughter, products available per species or production type, and especially production structures (7–9).

Antimicrobial sales data are defined as the minimal standard for monitoring programs by the World Organization for Animal Health [Office International des Epizooties, OIE; (10)]. They can be collected at either the manufacturer, wholesaler or pharmacy level depending on the national distribution routes of the products. Sales data are useful to evaluate long-term trends but do not include information about dose, route of administration, indication or duration of therapy. However, in the context of resistance epidemiology, only data about actual use of antimicrobials collected either at prescription or patient level might deliver the information necessary to establish and evaluate implemented measures. Such data can only be currently collected in few countries with advanced collection systems, such as Denmark (4) and The Netherlands (11) among other European countries. The AACTING network is maintaining a list of the various collection systems already in place (www.aacting.org). The collection of data at animal level is the ultimate goal of antimicrobial monitoring systems and, until this is available in all participating countries, alternatives using normalization of sales data by the total weight of the food producing animal population as a denominator have been developed. One such denominator is the population correction unit of the ESVAC project (12). Other institutions (13) and countries, including Canada (14) and Switzerland [ARCH-Vet; (3)], have implemented similar methods in their surveillance systems. As usage of antimicrobials is strongly dependent on population structure and repartition between high and low-using species, the normalization by weight may provide information on long-term trends but at the same time, a higher usage in one species will be "diluted" by lower usage species/production types (like dairy cows) with a large contribution to the overall livestock biomass (15). It is therefore important to measure antibiotic consumption as near as possible to the end users, i.e., to obtain information on species, dosage, duration and whenever possible, indication. The extrapolation of sales data using course doses is an interim measure to data collection at animal level. Course dose indicators have been proposed, such as the Animal Course Dose (ACD) by the French Agency for Food, Environmental, and Occupational Health & Safety (16) or Defined Course Dose (DCDvet) from the EMA (17). An advantage of ACD is its product-specific calculation, therefore better representing national specificities than DCDvet units. ACDs are established for each product using data from the summary of product characteristics (SPC) and contain the necessary detail on both dose and therefore potency, and duration of use.

The main aim of this study was to provide for the first time an extrapolation of the available national sales data to the number of treated animals in Switzerland. We chose to specifically investigate the treatment of pigs and calves because these are mainly reared and treated in groups via oral application. Due to the lack of detailed data about repartition of sales, we made assumptions regarding weight at treatment and repartition of sales data between species using a previously published repartition method. We then defined ACD for each product containing antimicrobials authorized in Switzerland for use in either pigs or calves and combined this information with national antibiotic sales data to extrapolate the number of potentially treated animals during the years 2011 to 2015.

MATERIALS AND METHODS

Data Collection

Veterinary antibiotic sales data for the years 2011 to 2015 were obtained from the Federal Office of Food Safety under a confidentiality agreement. Since 2004, sales data are collected in Switzerland from marketing authorization holders based on Article 35 of the Ordinance of Veterinary Medicines (18). Marketing authorization holders are required to deliver data on every product containing antimicrobials that was sold during a calendar year. Products subject to data collection are defined by their ATCvet codes (19) as listed in the ESVAC project (12). Additionally, data on antibiotic products not considered by the ESVAC project, like sprays or products to treat sensory organs, are also collected. Data obtained from the Federal Office of Food Safety for this study contained the quantity of active antimicrobial ingredient sold in kilogram for each product and year under investigation.

Animal Populations, Animal Weights, and Species Repartition

The amount of antimicrobials sold of products authorized for a single species was directly assigned to their target species. For each product authorized for more than one species, a repartition had to be determined. We used two distinct methods: the first one was used for premixes, the latter being legally defined in Switzerland as being "veterinary medicinal products used to treat

groups of animals and incorporated into either water or feed" [Ordinance on authorizations for medicinal products, Art. 2; (20)]. For all of these products, periodic safety update reports (PSURs) containing data on species repartition submitted to Swissmedic, the Swiss Agency for Therapeutic Products, during the years 2007 to 2012, were used. As premixes represented only 28 products from a total of 112 under investigation but between 57.6% (2015) and 67.8% (2011) of the total sales, another repartition method had to be used for oral solutions, oral powders and injectables. This repartition was done according to biomass repartition as described by Carmo et al. (21). Briefly, for each product authorized for one or more target species, each target species was assigned a percentage of kg of the total sales representing the proportion of its biomass in the total biomass of the list of authorized species for the product. For the present study, food producing animal population numbers were obtained from the Federal Office of Statistics (www.bfs.admin.ch), number of dogs from the ANIS database (Identitas AG, Bern, www.anis.ch) and the number of cats from the Swiss Association of pet food producers (Verband für Heimtiernahrung, Bern, www.vhn.ch). In analogy with calculations of the population correction unit (PCU) of ESVAC (12), the number of slaughtered animals were used for fattening pigs and calves, whereas data for dairy cows, sows, sheep, goats, horses, dogs, and cats represent live animals. Throughout the text and in the tables, "pigs" refer to fattening pigs.

Supplementary Table 1 lists the number of animals and the weights used for the biomass repartition. The most likely weight at treatment was sourced from the ESVAC report (12, 22). As heavy animals with a rather low treatment intensity, like dairy cows, skew the biomass repartition, we chose to only include them in the calculation when they were either explicitly listed as authorized species ("dairy cows") or, when a withdrawal time for milk was given in the SPC of the product. For pigs, we did not include the production stage of piglets or weaners. Using the number of animals in different production stages presents some challenges, the most prominent one for pigs being the lack of available data for the repartition of use between piglets and e.g., fattening pigs. Only few antimicrobials are primarily used in piglets or weaners, colistin being such an example. For almost all other products authorized for pigs, no data are available to stratify antimicrobial consumption per different age classes using sales data. Repartition data will only be available once reporting of all treatments with antimicrobials in Switzerland is made mandatory at the end of the year 2019. For this reason and because sales data include the use of antimicrobials by all the age categories of the species for the years under investigation, we used slaughtered numbers of pigs as denominator for the therapeutic incidence in this species. Finally, for injectable products authorized without indication of the production stage ("bovines" containing dairy cows and "pigs" representing slaughtered pigs and sows) we used raw data provided by experts for the study by Carmo et al. (23) to determine if a use would take place in the particular production stage under consideration.

Calculation of Animal Course Doses and Therapeutic Intensity

The Animal Course Dose (ACD) was calculated for each active pharmaceutical ingredient contained in each product authorized during the years under investigation. Data were collected from the authorized summary of product characteristics (24) and entered into an MS Excel sheet containing: name of the product, authorization number, list of authorized species, active ingredient(s), dose and duration. Doses given in international units were converted to mg using conversion factors listed in the ESVAC report (12). Whenever the recommended dose was a range, the highest recommended dose and longest duration were chosen to reflect the minimal number of animals potentially treated. Moreover, when different doses were authorized for different indications, the most likely indication was chosen. This was the case for products presenting both a prophylactic or metaphylactic indication with different doses and duration. ACDs were defined per kg and the ACD per animal obtained by multiplication with the likely weight at treatment. To take Swiss specificities into account, the weight at treatment for pigs was taken from a previous study by Schnetzer et al. (25) and the weight for calves based on expert opinion (Prof. M. Kaske, Zurich, personal communication).

Therapeutic intensity reflects the number of ACDs per slaughtered animal (pig or calf) in 1 year. For combination products, the number of ACDs was calculated separately for each active pharmaceutical ingredient. Therefore, a single treatment with a combination containing 3 antimicrobials results in 3 ACDs. ACD and intensity were calculated using the following equations:

$$Number\ of\ ACDs = \frac{total\ quantity\ of\ active\ ingredient\ sold\ in\ one\ year\ (mg)}{daily\ dose\ \left(\frac{mg}{kg}\right) \times\ duration\ of\ tratment\ \left(days\right) \times\ weight\ at\ treatment\ (kg)}$$

$$Therapeutic\ intensity\ in\ species\ X = \frac{Number\ of\ ACDs\ in\ species\ X}{Total\ number\ of\ animals\ for\ species\ X}$$

RESULTS

From the year 2011 to 2015, sales of antibiotics for use in food producing animals decreased by 29.8% (**Table 1**). In the same time, the percentage represented by premixes decreased from 67.8 to 57.7%. Therefore, measured in kg, antimicrobials sold in premixes made the largest part of yearly sales of antimicrobials for the veterinary medicine. As a consequence, pigs and calves are the most pertinent species among food producing animals to be investigated for use and trend detection. In tonnage sold for use in these species, the decrease in the 5 years under investigation is comparable: 38.4% in pigs and 30.1% in calves. However,

TABLE 1 | Sales, biomass and mg per kg biomass for food producing animals as well as pigs and calves for the years 2011 to 2015.

	2011	2012	2013	2014	2015
ALL FOOD PRODUCING ANIMALS					
Sales (kg)[a]	58,942	54,169	50,370	46,147	41,378
% Premixes	67.8	65.7	64.5	61.9	57.7
mg/kg	72	66	62	56	51
PIGS					
Sales (kg)	22,475	20,276	18,890	16,458	13,845
mg/kg	121.8	112.5	108.1	92.0	77.4
CALVES					
Sales (kg)	21,293	19,299	17,941	16,385	14,886
mg/kg	582.1	537.5	508.3	465.3	449.9

[a]*Sales data detailed by antimicrobial classes for the years under investigation are available under https://www.blv.admin.ch/blv/de/home/tiere/publikationen-und-forschung/statistiken-berichte-tiere.html.*

TABLE 2 | Number of estimated ACDs per pigs or calves, oral and parenteral application, from 2011 to 2015.

	2011	2012	2013	2014	2015
PIGS					
Number of ACDs	8,663,191	7,686,268	7,184,114	6,674,046	5,914,349
Number slaughtered	2,839,106	2,773,726	2,689,576	2,751,721	2,753,256
Intensity[a]	3.051	2.771	2.671	2.425	2.148
CALVES					
Number of ACDs	1,828,599	1,687,942	1,636,930	1,521,050	1,407,450
Number slaughtered	261,308	256,471	252,118	251,509	236,343
Intensity[a]	6.998	6.581	6.493	6.048	5.955

[a]*Number of ACDs per slaughtered animal.*

normalizing these numbers to the respective biomass of the produced (slaughtered) population reveals a much higher use per kg of biomass for calves (449.9 mg/kg biomass in 2015) than for pigs (77.4 mg/kg biomass). The difference between both species even increased from 4.8-fold higher for calves in 2011 to 5.8 in 2015.

Normalizing sales data to either the overall biomass of food producing animals or to the biomass of a particular species is a crude estimate of antimicrobials use, not taking dose or duration into account. We therefore calculated the number of course doses (ACDs) per product and species. A summary of the results is presented in **Table 2**. The total number of ACDs was approximately 4.5 times higher in pigs and decreased by 31.7% over the years under investigation, whereas the decrease for calves was 23.0%. Normalization to the number of slaughtered animals showed a much slower decrease of 14.9% for calves between 2011 and 2015 compared to 29.6% in pigs. As a result, the difference between both species grew from 2.3-fold in the year 2011 to 2.8-fold in the final year under investigation.

Not all antibiotics have the same potential impact on resistance selection and consequences for the treatment of both humans and animals. Moreover, different products are authorized for distinct conditions in pigs or calves. The repartition of the number of ACDs per class of antimicrobials was therefore calculated separately for each species for the year 2015. **Table 3** presents the repartition by antimicrobial class for ingredients sold in premixes and as parenteral injections. In this year, polymyxins (in form of colistin) were the class with the highest potential numbers of ACDs per pigs, followed by sulfonamides. In calves, the highest number was represented by penicillins (mainly sold as aminopenicillins) followed by tetracyclines. The total number of ACDs per animal was 4.43 times higher in calves than in pigs. The same calculation was done for injectable products as these may contain antimicrobials of the highest priority [HPCIA; (26)] not available for oral application. For pigs, the highest number of ACDs per animal in the year 2015 was represented by macrolides, followed by aminoglycosides and fluoroquinolones. For calves (amino)penicillins were the class with the highest number of course doses per animal, followed by macrolides and aminoglycosides. The total number of potential ACDs per animal for injectable products in the year 2015 was 1.485 for calves and 1.088 for pigs.

TABLE 3 | Number of estimated ACDs per animal, antimicrobials administered as premix or parenterally in pigs and calves for the year 2015, presented by classes of antimicrobials.

Classes of antimicrobials	Pigs		Calves	
	Parenteral	Premixes	Parenteral	Premixes
Sulfonamides	0.050	0.177	0.042	0.632
Penicillins	0.389	0.155	0.477	1.324
Tetracyclines	0.069	0.155	0.057	1.069
Aminoglycosides	0.132	–*	0.210	–*
Amphenicoles	0.054	–*	0.098	–*
Macrolides	0.170	0.145	0.303	0.862
Cephalosporins, 3rd and 4th generation	0.061	–*	0.112	–*
Fluoroquinolones	0.122	–*	0.194	–*
Polymyxins	–*	0.189	–*	–*
Total	1.088	0.889	1.485	3.942

**No products authorized for the combination of class, species and application route.*

Finally, the evolution of the number of potential ACDs per animal for HPCIAs is presented in **Table 4**. For macrolides used in pigs, a decrease of 22.0% for products sold as premixes was attenuated by a corresponding increase of 11.8% for injectables. This pattern was even more evident in calves where a reduction of 27.1% for premixes was almost completely compensated by an increase of 25.7% in injectables. With respect to the other two classes of HPCIAs, sales of products containing fluoroquinolones remained stable for pigs (−1.5%) and an increase of 6.4% was observed for the number of potential ACDs per animal in calves. Courses with cephalosporins of the third and fourth generations showed a comparable decrease in pigs (−16.4%) and calves (−15.7%).

TABLE 4 | Number of estimated ACDs per animal, macrolides, fluoroquinolones and cephalosporins 3rd and 4th generation, for pigs and calves, by administration route.

	2011	2012	2013	2014	2015
PIGS					
Macrolides, premix	0.186	0.199	0.191	0.160	0.145
Macrolides, injections	0.152	0.153	0.171	0.165	0.170
Fluoroquinolones*	0.128	0.115	0.142	0.136	0.126
Cephalosporins*	0.073	0.062	0.064	0.067	0.061
CALVES					
Macrolides, premix	1.183	1.087	0.998	0.891	0.862
Macrolides, injections	0.241	0.239	0.291	0.287	0.303
Fluoroquinolones*	0.156	0.145	0.171	0.165	0.166
Cephalosporins*	0.133	0.123	0.121	0.119	0.112

*Only available as injections.

DISCUSSION

This is the first study at national level using the ACD concept applied to sales of antimicrobials with the objective of extrapolating the number of potentially treated pigs and calves in Switzerland. Sales of antimicrobials for the veterinary medicine are published at national level since 2005. So far, these data represent the only available source of exhaustive antimicrobial consumption data at national level. Sales figures may allow for the recognition of trends, but the lack of information on potency, dose, duration of treatment and repartition per species strongly limits their usefulness. The indicator ACD may therefore help to bridge that gap. Calculation of ACD and repartition of quantities for products authorized for more than one species would not be possible without making assumptions, which might influence the results. The first assumption relates to the weight of the animals. The standard weight has an impact on both the calculation of the species repartition and the ACD indicator itself. The impact of using different weights is a topic beyond the scope of this study and the impact on calculations has been studied elsewhere (27–29). In this study we used weights at treatment as close as possible to the Swiss reality. This should provide the best fitting results, and also guarantees future reproducibility of the method and comparison of results, as these weights are likely to be used when quantifying Swiss antimicrobial consumption both at national and international level. This approach is comparable to the one chosen by the ESVAC project.

The method used to stratify antimicrobial consumption by the production types included in the study has some potential bias. As it is based on the total biomass of each animal category, the resulting estimates are highly dependent on the animal demographics and the animal average weight used. This might not always be a representative surrogate of the product repartition by each category. As a reliable repartition is generated by data collected on actual usage, and such data are currently not available in Switzerland, we chose an alternative that was applicable at product level that would deliver reproducible results over the years and be as accurate as possible. Carmo et al. (21) have compared three different methods to determine species repartition of antimicrobials. The longitudinal study extrapolation method (based on field data) was not applicable at single product level due to the requirement for minimum, mode, and maximum starting values. The biomass distribution was shown to be the method providing the closest results to the extrapolation based on field data, thereby increasing our confidence on the pertinence of the approach we applied. The two main drawbacks of this method are the dependence on defined average weights and country specific animal demographics. However, the method, limited by the data available in the current Swiss context, provides a first insight into antimicrobial consumption patterns in different species/production types. In the future, the data collection system IS-ABV (description available under http://www.aacting.org/matrix/is-abv/?lid=1447) shall provide further insights into these patterns, as well as a basis for comparison with the results from the method and its potential biases. To make our extrapolations as comparable as possible with other projects, we used the same standard weights as in the ESVAC project (12).

It must also be noticed that the denominators of the indicators presented were based on the number of slaughtered animals only. The weights used for the calculation of the biomass were likely weights at treatment as defined in the ESVAC Project (12). The use of such a calculation might hinder direct comparisons with other studies and should be taken into consideration when benchmarking these results. When using the biomass as a denominator, the result should be interpreted as an indicator for the amount of active ingredient used per kg of animal produced. Likewise, the therapeutic intensity indicates the average number of ACDs per animal produced/slaughtered.

Both a high proportion of heavier animals like cows or, alternatively, a high treatment intensity in a species of lower biomass are examples of how animal demographics can bias the results of the stratification approach based on the biomass. The repartition across species is mainly influenced by national production structures. In Switzerland, dairy production is an important agricultural sector and therefore dairy cows make a high proportion of the food producing animals' sector (15). Cows represented 49% of the total biomass in the year 2015 and this high proportion leads to an underestimation of the repartition of sales for pigs or calves. This primarily affects the repartition of aminoglycosides and cephalosporins of the third generation, which are antimicrobials frequently used in the treatment of dairy cows. The calculated numbers of ACDs per animal for these classes presented in **Table 4** are, therefore, an underestimation. Within the same species, biomass repartition could have been used to estimate the use of antimicrobials in different production stages of pigs. However, using piglets, weaners and fattening pigs produced during 1 year introduces the bias of counting a significant but undefined proportion of the animals two or three times. As sales data were only available for one full year, we therefore chose to base our repartition, as well as the denominator for the treatment intensity, on the number of pigs slaughtered during the same year. This indicator is used in this study as a surrogate for all pig production stages.

As the numbers of ACDs represent an extrapolation of usage data based on sales figures, they follow the latter closely. The

downward trends in sales is mirrored by the treatment numbers of both calves and pigs. However, differences become evident as soon as additional factors like application route are taken into account. The repartition for pigs in the year 2015 shows that 18% of the active ingredients were used parenterally when based on quantity, whereas they represented 51% of the treatments when using ACDs. The main reason for this difference lies in the potency of the active ingredients: antimicrobials are used parenterally with a lower dose as there is no loss of active ingredient compared to the lower bioavailability following oral application. Another possible reason is the use of more than one ACD for parenterally applied combination products as 12 of 71 injectable products investigated were combinations of two active ingredients. Whereas, this approach can be disputed as it shows a higher number of "treatments," we think that the use of ACDs is better suited to test for associations between antimicrobial use and resistance.

Converting sales of antimicrobials to number of treatments per animal allows detection of trends that would not be obvious when only assessing the quantity of active ingredients sold. Macrolides used to treat calves provide a good example: our results show a clear shift from the oral application in form of premixes toward an increase in the use of injectables. One possible explanation is the increasing availability of macrolide antibiotics with a long duration of action, e.g., tulathromycin, tildipirosin, and gamithromycin. Such active compounds combine the easy use of a single application with a long action. Moreover, for parenteral applications, both time to maximal concentration and maintenance of active levels are not influenced by the appetite of the animals, therefore guaranteeing the adequate treatment of sick animals with reduced appetite. On the negative side, studies about macrolides used in human medicine convincingly showed a higher level of resistance selection for longer acting molecules (30).

Our results show a strong difference in the extrapolated usage of antimicrobials in pigs and calves. This cannot be explained by a single factor as the administration of antimicrobials is driven by medical, economic and also psychosocial factors. Crowding effect, stress during transport of very young, not yet immunocompetent animals, partially inadequate colostrum feeding and less than ideal stable climate are among the factors favoring respiratory problems in calves (31, 32). In the swine industry, some of the abovementioned factors also exist, but the structure and management of pig production limits the risks. Management practices like all-in-all-out including disinfection between the batches or integrated production from piglet to finisher can strongly help to reduce antimicrobial usage. In pigs, there are two main periods at risk for treatment with antimicrobials: the first at weaning with around 12 kg body weight and the second at around 25 to 29 kg body weight (25, 33). In pigs, diarrhea is one of the leading indications for treatment. This is a very unspecific symptom with many different causes, including not only bacterial but also dietary or viral origins. In this context, the availability of vaccines against both circovirus and *Lawsonia intracellularis* infections in the years 2008 to 2010 contributed to the reduction of diarrheal symptoms, and hence, the rather indiscriminate use of antibiotics to treat such symptoms. For calves, respiratory diseases are much more multifactorial and the introduction of various vaccines (against bovine respiratory syncytial, parainfluenza or corona virus) seems not to have had the same positive effect as in the pig industry.

Several factors hinder a proper comparison of our results with previously published data. To the best of our knowledge, this is the first time that the ACD indicator is used at national level in Switzerland. As a matter of fact, its use is not currently widespread in other countries, with the exception of France where it was developed. However, the comparison with French data is difficult. No publication presents the French antimicrobial consumption using ACDs per animal and year as an indicator. The French indicator for exposure to antimicrobials is ALEA [Animal Level of Exposure to Antimicrobials; (16)]. It is obtained by dividing the effectively treated biomass by the total biomass of the same species. The global ALEA calculated for the year 2015 in France was 0.488 and represented a decrease of 20.1% compared to 2011. Another difficulty is the use of different production categories and standard weights at treatment. For pigs, the French system uses weights up to 350 kg for a specific category of sows and the average for the pig population is set at 105 kg. This is 3.62 times higher than the standard weight at treatment of 29 kg identified in previous Swiss studies and used here. The differences in the standard weights at treatment also explain the discrepancies in the antimicrobial consumption for France published, for the same year, in the ESVAC report (107 mg/PCU) and in the ANSES report (47 mg/kg). Due to the differences in weight and in the categories, and the difficulties in making assumptions and extrapolations, we decided not the compare our figures to the French ones.

Our data can only be compared with countries where calves are reared for the production of veal meat. Besides France and Belgium (for which country we could not find adequate data for comparison), this production system also exists in The Netherlands. The available report for the year 2015 (34) uses indicators differing from the ones in the present study but still shows a higher treatment intensity in calves compared to pigs. This is in line with the present study, where the antimicrobial use was 2.77-fold higher in calves than in pigs.

Both examples clearly illustrate the need to harmonize methodologies at international level in order to discuss data collected in different countries. Such discussions currently take place within the AACTNG network (www.aacting.org).

CONCLUSION

This first study of the number of treatments of pigs and calves extrapolated from yearly sales shows both similarities and differences between the two species under consideration. Whereas, the sales by species and the number of extrapolated treatments both decreased in a similar way, the difference in the number of treatments per animal between pigs and calves differed over the years under investigation. Given that the applied method is based on the extrapolation of sales figures, a similar decrease at species level was to be expected. However, the use of

course doses allows to further investigate trends in the patterns of antimicrobial treatments. In our study, this was very clear for the class of macrolides, for which the decreases in oral use were partly (pigs) or completely (calves) compensated by the application of long acting injectables. We, therefore, recommend the use of extrapolated treatment numbers when no exhaustive collection of usage data is in place. The concept of ACDs can also complement the collection of antimicrobial consumption data at species level allowing for their validation using sales data.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the manuscript/**Supplementary Files**.

AUTHOR CONTRIBUTIONS

RS did all the calculations presented in this work. LC helped with the repartition of sales between species, expert advice and biomass distribution. Raw sales data and advice regarding their use was provided by DH. The study was designed by CM and supervised by KE and HN.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2019.00318/full#supplementary-material

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METHODS
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OIE Annual Report on Antimicrobial Agents Intended for Use in Animals: Methods Used

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For over two decades, the World Organisation for Animal Health (OIE) has engaged in combatting antimicrobial resistance (AMR) through a One Health approach. Monitoring of antimicrobial use (AMU) is an important source of information that together with surveillance of AMR can be used for the assessment and management of risks related to AMR. In the framework of the Global Action Plan on AMR, the OIE has built a global database on antimicrobial agents intended for use in animals, supported by the Tripartite (World Health Organization (WHO), Food and Agriculture Organization of the United Nations (FAO) and OIE) collaboration. The OIE launched its first annual data collection in 2015 and published the Report in 2016. The second Report, published in 2017, introduced a new methodology to report quantitative data in the context of relevant animal populations, and included for the first time an annual analysis of antimicrobial quantities adjusted for animal biomass on a global and regional level. A continuing annual increase of countries participating in the data collection demonstrates the countries engagement for the global development of monitoring and surveillance systems in line with OIE international standards. Where countries are not yet able to contribute their quantitative data, their reports also highlight the barriers that impede them in data collection, analysis and/or reporting. The OIE Reports show annual global and regional estimates of antimicrobial agents intended for use in animals adjusted for animal biomass, as represented by the quantitative data reported by countries to the OIE. The OIE advises caution in interpretation of estimates made in the first few years of reporting recognizing some important limitations faced by countries as they develop their monitoring systems. The OIE remains strongly committed to supporting its Members in developing robust and transparent measurement and reporting mechanisms for AMU.

Keywords: antimicrobial resistance (AMR), antimicrobial use (AMU), report, methods/methodology, surveillance, monitoring

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INTRODUCTION

The World Organisation for Animal Health (OIE) has worked actively for more than two decades on veterinary products, including antimicrobial agents, and developed a coherent strategy for its activities in this area (1). Monitoring of antimicrobial use (AMU) is an important source of information that, together with surveillance of AMR, can be used for the assessment

and management of risks related to AMR. Toward standardization of surveillance and monitoring data worldwide, the OIE developed standards on "Monitoring of the quantities and usage patterns of antimicrobial agents used in food producing animals" [(2) Terrestrial Animal Health Code Chapter 6.9.], "Monitoring of the quantities and usage patterns of antimicrobial agents used in aquatic animals" [(3) Aquatic Animal Health Code Chapter 6.3.] and on the "Harmonization of national antimicrobial resistance surveillance and monitoring programmes" [(2) Terrestrial Animal Health Code Chapter 6.8.], and "Development and harmonization of national antimicrobial resistance surveillance and monitoring programmes for aquatic animals" [(3) Aquatic Animal Health Code Chapter 6.4] (3).

In the framework of the Global Action Plan on AMR, the OIE has also built a global database on antimicrobial agents intended for use in animals, supported by the Tripartite collaboration (WHO, FAO, OIE).

The OIE *ad hoc* Group on Antimicrobial Resistance developed a template for harmonized AMU data collection, as well as guidance for its completion that are available in the three official OIE languages (i.e., English, French, and Spanish) (4).

The OIE launched its annual data collection on AMU in 2015, and published the first Report in 2016 (5).

The second Report, published in 2017, introduced a new methodology to report quantitative data in the context of relevant animal populations and included for the first time an annual analysis of antimicrobial quantities adjusted for animal biomass on a global and regional level (6). The third report using the same methodology was published in February 2019 (7). The OIE animal biomass methodology was developed with the goal of best representing animal biomass in all OIE Regions, with different animal populations and production systems, and data collection systems, using the data available at the international level.

The methodology for the animal biomass calculation was developed with the support and validation of the OIE *ad hoc* Group on Antimicrobial Resistance, and shared with Member Countries in the report of the OIE Scientific Commission for Animal Diseases meeting of September 2017.

The methodology for calculating animal biomass on a global level, used by the OIE for analysis of reported data on antimicrobial agents intended for use in animals, is detailed in this article.

MATERIALS AND METHODS

OIE AMU Data Collection

Each year in October, the template and accompanying guidance documents are sent to all 182 OIE Member Countries and 11 non-OIE Member Countries. The deadline for submission is December, but responses may be accepted until mid-May of the following year.

The template, to be completed by the respondents, is provided in the form of an Excel file that includes four worksheets labeled "Baseline Information," "Reporting Option 1," "Reporting Option 2," and "Reporting Option 3."

The "Baseline Information" sheet can be answered by any country, and collects general information on topics including the use of antimicrobials as growth promoters, and any barriers to reporting quantitative data on antimicrobial agents used in animals.

For countries able to provide quantitative data on antimicrobial agents intended for use in animals, the Baseline Information sheet also contains questions relevant to data collection such as data sources, year and animal species covered by the reported data.

Following completion of the Baseline Information, the template either directs countries to submit the questionnaire if no quantitative data are available, or to complete one of the three "Reporting Options" if quantitative data are available. The three reporting options represent increasing levels of detail of quantitative data on antimicrobial classes used in animals, with the possibility of separating amounts reported by type of use ("veterinary medical use," which includes use to treat, control, or prevent disease; and "non-veterinary medical use," which includes use for growth promotion), animal groups (terrestrial food-producing, aquatic food-producing, or companion) and routes of administration.

Antimicrobial Agents Reported

For the harmonization of the submitted data, the OIE established the List of antimicrobial classes to be reported by the participant countries (**Table 1**). Data on antimicrobials sold/imported/prescribed/used in the country in animals are reported at the class/subclass level. All pharmaceutical forms are included. The quantities for each antimicrobial class can be reported either for veterinary medical use (including prevention of clinical signs) or for growth promotion purpose.

If there are confidentiality or proprietary reason that imped a country to individually report the quantities for one or more antimicrobial classes, such quantities should report as "Aggregated class data," an existing category in all three Reporting Options proposed by the OIE. The country that uses this category should list the names of the antimicrobial classes that cannot be reported individually.

For each cycle of data collection, a specific year is targeted—for example, data from 2015 for the third report published in 2019. However, countries with more recent quantitative data may also report that data.

For each reported year, the country informs the OIE on the period of time covered within the year, the data sources (**Table 2**), coverage of the data (if is <100% the country explains which quantitative data is inaccessible), animal groups covered (terrestrial food-producing animals, aquatic food-producing animals, and companion animals), food-producing species covered, species considered companion animals covered by the data and the link to the national report available on the web, if any.

Calculation of Kilograms of Active Ingredients

For the purpose of reporting data on antimicrobial quantities (amounts sold or imported for use in animals expressed in kilograms (kg) of antimicrobial agent, i.e., chemical compound as declared on the

TABLE 1 | Classes of antimicrobial agents for reporting.

Antimicrobial class	Guidance
Aminoglycosides	Includes aminocyclitols (e.g., streptomycin, dihydrostreptomycin, and spectinomycin) and all other aminoglycosides (e.g., gentamicin, kanamycin, neomycin, apramycin).
Amphenicols	Includes florfenicol and thiamphenicol.
Arsenicals	Includes nitarsone, roxarsone, and others.
Cephalosporins	May be reported as, • Cephalosporins (all generations) or • In relevant category groupings: ○ 1–2 generation cephalosporins and ○ 3–4 generation cephalosporins
Fluoroquinolones	Includes danofloxacin, difloxacin, enrofloxacin, marbofloxacin, and other fluoroquinolones, but not other quinolones (e.g., flumequine, oxolinic acid, nalidixic acid), which are reported separately.
Glycopeptides	Includes avoparcin and others.
Glycophospholipids	Includes bambermycin (i.e., flavomycin).
Lincosamides	Includes lincomycin, pirlimycin, and others.
Macrolides	Includes substances with all macrolide structures, such as erythromycin, spiramycin, tylosin, tylvalosin, gamithromycin, tildipirosin, tulathromycin, and others.
Nitrofurans	Includes furazolidone, nitrofurantoin, nitrofurazone, and others.
Orthosomycins	Includes avilamycin and others.
Other quinolones	Includes flumequine, nalidixic acid, oxolinic acid, and others.
Penicillins	Includes all penicillins (e.g., natural penicillins, aminopenicillins, and others), but excludes other beta lactam antimicrobials like cephalosporins.
Pleuromutilins	Includes tiamulin, valnemulin, and others.
Quinoxalines	Includes carbadox, olaquindox, and others.
Streptogramins	Includes virginiamycin, pristinamycin, and others.
Sulfonamides (including trimethoprim)	Includes all sulfonamides, as well as trimethoprim, and similar compounds.
Tetracyclines	Includes chlortetracycline, doxycycline, tetracycline, and oxytetracycline.
Others	All others not covered, including coumarin antimicrobials, e.g., novobiocin, fusidic acid, kirromycins, phosphonic acids like fosfomycin, rifamycins, thiostrepton.

TABLE 2 | Data Sources proposed by the OIE.

Sales data	Wholesalers
	Retailers
	Marketing authorization holders
	Registration authorities
	Feed mills
	Pharmacies
	Farms shops/agricultural suppliers
	Industry trade associations
Purchase Data	Wholesalers
	Retailers
	Feed mills
	Pharmacies
	Agricultural cooperatives
	Producer organizations
Import data	Customs declarations—veterinary medicinal product
	Customs declarations—active ingredients
Veterinary data	Sales
	Prescriptions
Antimicrobial use data	Farm records
Other data source(S)	Other—free text field

product label, that is to be calculated from the available information), animals are grouped into "all animal species," "companion animals," "all food-producing animals," "terrestrial food-producing animals," and "aquatic food-producing animals."

The amount of the antimicrobial agents intended for use in animals in kilograms (kg) (the chemical compound or active ingredient as declared on the product label, that is to be calculated from the available information) should be reported. Where data are available in the form of number of packages of a given pharmaceutical preparation sold/imported/prescribed/used; international units (IU) and; percentage weight per volume (% w/v), mathematical conversion are necessary in order to report the kilograms of active ingredients to the OIE.

Ideally, the OIE is interested in the amount of active ingredient (moiety), that is, the substance as listed in the *OIE List of antimicrobial agents of veterinary importance* (8) (e.g., benzylpenicillin), not the total weight of the actual chemical compound (salt, ester, or other, for example: sodium or potassium benzylpenicillin) contained in a veterinary medicinal product or traded as bulk material. At this stage of the project, the precision gained by the refined reporting of amounts of active ingredient, achieved by mathematical conversion of amounts of chemical compound as declared on the product label, is not justified. Therefore, the OIE template accepts the amounts of chemical compound as declared on the product label.

Since the second year of data collection, a question was added to the template in order to understand the barriers impeding countries from reporting amounts of antimicrobial agents in animals. This information is useful for guiding discussion on overcoming barriers during training Seminars of National Focal Points for Veterinary Products (those who most frequently complete the OIE Template) and increasing availability of quantitative data in the future, and reflects challenges in National Action Plan implementation that would also be assessed during the Performance of the Veterinary Services (PVS pathway) evaluation.

The barriers highlighted by responding countries are grouped into four main categories (Lack of regulatory framework, Lack of coordination/cooperation between national authorities and with private sector, Lack of tools and human resources, and Insufficient regulatory enforcement).

Validation of the Data and Calculations Performed at the OIE Level on Antimicrobials Quantities

The OIE systematically reviews the reported information and systematically comes back to responding countries to clarify some issues, or to request missing data.

Whenever possible, the data reported by countries are checked by the OIE against existing reference sources, using the previous year's reported data and/or national reports available online. The indicator for this comparison is a calculated "percentage of change" relative to the reference.

In the countries with high percentages of unexplained change ($> \pm 25\%$), the OIE inquires how the calculations to obtain kg of antimicrobial agents sold/used were carried out. Through this process, errors in the calculations can be identified and corrected.

When all the responses have been validated, the OIE proceeds with analysis toward preparation of the report. The amounts of antimicrobials sold/used are calculated by country, by region, by animal groups, type of use, and pharmaceutical forms.

As all the countries do not provide the same level of details, some calculations are done on only a part of the countries providing quantitative data.

Through this process, the final result is a quantity of antimicrobials sold/used expressed in kg/country/year. As many countries are still in the process of developing their data collection systems, these results are reported by the OIE on the global and regional level at this stage.

OIE Calculation of the Animal Biomass

To compare quantitative data reported on antimicrobial agents intended for use in animals between regions and over time, a scale is necessary to evaluate these data in the context of the relevant animal populations, which vary in size, and composition.

Therefore, the quantitative data reported on antimicrobial agents intended for use in animals needs to be adjusted for the relevant animal biomass according to the following calculation:

$$\frac{antimicrobial\ agents\ reported\ (mg)}{animal\ biomass\ (kg)}$$

Animal biomass is calculated as the total weight of the live domestic animals in a given population present during a year in a specific area, used as a proxy to represent those likely exposed to the quantities of antimicrobial agents reported.

Animal biomass is currently employed as a denominator in analysis of quantitative antimicrobial use data by other national and regional antimicrobial use surveillance groups, such as the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC)—(9), the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS)—(10), and the Japanese Veterinary Antimicrobial Resistance Monitoring System (JVARM, (11)). In 2017 the US FDA proposed a method for estimating a Biomass denominator (12).

While several methodologies have been developed for the calculation of animal biomass by other surveillance groups, none could be directly used for the OIE global database on antimicrobial agents intended for use in animals. Particularly, these methodologies utilize available data on animal populations detailed by production class, estimates of live animal weights, import/export data, and total annual populations of production groups living <1 year (i.e., poultry, veal calves, fattening pigs, lambs, and kids). On a global level, such detailed data are not yet available for many countries, and therefore a new methodology was developed by the OIE mainly using globally available datasets—the OIE World Animal Health Information System (WAHIS) and the United Nations Food and Agriculture Organization Statistics (FAOSTAT).

The formulas for calculating biomass by species were developed using the two globally available datasets, WAHIS and FAOSTAT, and were compared to references from countries where more detailed animal population data by production class were available. These references include animal biomass figures either directly supplied from Member Countries, or calculated from animal population data in Eurostat, the statistical office of the European Union.

The formulas chosen for calculation of the OIE denominator reflect the best fit estimations using the more general global animal population data (WAHIS, FAOSTAT) when compared to these available reference figures. The derived formulas were then applied to all countries providing quantitative data for the target year.

All weights and biomass figures are measured in kilograms (kg).

Data collected by these global animal surveillance databases, WAHIS and FAOSTAT, are point in time species-level census data[1] with little to no detail on the production class. Such data are difficult to interpret given that production classes within a species can have very different average weights, such as beef cattle and veal calves. Additionally, given that census data are collected at one point in time of the year, the total annual population is not known for production groups which are slaughtered and repopulated a certain number of times within 1 year (this multiplication factor is hereafter referred to as "cycle factor").

In development of the methodology for calculation of an annual animal biomass, the underlying effort was to best use globally available census data from the OIE WAHIS interface. WAHIS data are reported by National Veterinary Services through OIE Focal Points for Animal Disease Notification, and the figures are subsequently validated by OIE staff.

FAOSTAT animal population data are used as a complementary dataset. FAOSTAT data are similarly primarily obtained from national governments, but sources expand beyond National Veterinary Services to National Statistics Offices and other relevant agencies. When a national government does not report a figure to FAOSTAT, FAO uses local expert resources to estimate a figure, or their statistical team to imputate a data point. The two datasets are therefore similar but can display significant variation.

Where census data were used, the WAHIS and FAOSTAT figures are first cross-referenced with each other, and then with national reports or literature as needed. FAOSTAT data are used

[1] Point in time census data represents the number of living animals in a country at the time of survey.

when a WAHIS data point is not available or is outside of expected variation without explanation.

In addition to census data, FAOSTAT also reports numbers and tons of production animal species slaughtered by country each year, similarly undifferentiated by production class. As WAHIS does not collect this information, FAOSTAT slaughter data is used when these data were needed. For species living <1 year, it was necessary to use data on number of animals slaughtered to represent an annual population, as this information cannot be extrapolated from point in time census data without a cycle factor specific to each country's production model.

RESULTS

Principles of Animal Biomass Calculation Methodology

The overall objective of the methodology is to obtain the biomass of animals present during the year of analysis in a specific country using internationally available data.

For a given species, animal weight varies by age and production class, and therefore the structure of the population of a given species must be taken into account.

The first approach is to distinguish animals from production classes with a lifespan >1 year, and those with a lifespan of <1 year.

For animals living for more than 1 year, it was considered that census data (number of animals present at one time) can be a good basis to evaluate the number of animals present in the country during the year.

In this case, the biomass can be obtained by multiplying the number of animals present at one point in time (census data) by a calculated weight (if available or possible to calculate) or standard weight.

Generally, census data available represent the number of animals present at one point in time and include all animals within the species regardless of their age and production class. Thus, it is necessary to estimate the number of adults vs. young animals to ensure appropriate average weights were applied. Different methods for this estimation were used depending on the species and available data.

For example, to differentiate breeding swine (sows) with a production lifespan >1 year, from the fattening pigs, an estimation of the percentage of sows in the total pig population was calculated based on Eurostat data, where production-class detailed information is available. To calculate the number of sows, the percentage obtained was applied to the census data as a constant for all the countries. For cattle, the approach is different due to the large variation of body weight between production classes (calves, young cattle, and adults), and a broader diversity of production models (veal meat, beef meat, milking cows, etc…). For this species a model was built to estimate the structure of an average bovine population, based on Eurostat production-class information, and was applied to all the countries.

The mean weight of adult animals was generally based on existing standard weights, adapted regionally by livestock unit classification (13). For animals living <1 year, production data (number and weight of animals slaughtered annually) were considered as a good basis to estimate the average weight of animals present in the country during the year.

The application of these principles for calculating mean weights depends on the species; in some species, like poultry, mainly young animals are slaughtered (with a production life less than a year). In other species like cattle, goats, sheep, and swine, despite a large number of young animals being slaughtered, adults (with a production life greater than a year) may represent a significant portion of the population.

Calculation Methodology of Average Animal Weights

Different antimicrobial use surveillance programmes have used various methodologies for determining the average animal weights used in the calculation of total biomass.

In the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC), estimated average weights at time of treatment are used (9). The Canadian Integrated Surveillance Program for Antimicrobial Resistance (CIPARS) also uses the same estimated weights at time of treatment, as well as Canadian standard weights (10). The surveillance programs of Japan and the United States (12) take a different approach, instead using more general estimates of average animal weights by production category, rather than focusing the estimates on an average size at treatment.

On a global scale, it was not considered feasible to estimate weights at time of treatment for all countries reporting data to the OIE. The live weight of the animal before slaughter was most easily accessible, and was considered to be a best representation of average weights for a global calculation of animal biomass.

The live weights of animals before slaughter were calculated using FAOSTAT annual slaughter data, for all species and regions where these data were available, using the following two formulas:

$$carcass\ weight\ (kg) = \frac{total\ weight\ of\ species\ slaughtered\ (kg)}{number\ of\ species\ slaughtered\ (heads)}$$

Carcass weights were converted to live weights at time of slaughter using conversion coefficients (k) as defined by Eurostat, also known as dressing percentages (14). Conversion coefficients represent the difference between a processed carcass weight and the expected live weight of that animal species before slaughter, expressed as a fraction.

$$live\ weight\ (kg) = \frac{carcass\ weight\ (kg)}{conversion\ coefficient\ (k)}$$

Bovine (including cattle and domestic buffalo) biomass was calculated according to the following principles:

1. Countries were grouped by sub-region as defined by livestock unit classifications (13). A sub-regional mean live weight was then determined by calculating the average live weight of

bovines for countries within the sub-regional grouping from their production data;

2. From the calculated sub-regional mean live weight, the weights of the different bovine production categories [adults, young (between 1 and 2 years of age), calves (<1 year of age)] were determined by applying relevant weight proportions standards, originating from livestock unit ratios defined by Eurostat (15). Consecutively, the weight of each bovine production category was then multiplied by a predicted population ratio. These population ratios were calculated in the reference Eurostat database and consider an anticipated renewal rate of 30%.

Bovine biomass was calculated by multiplying the representative weight determined for each sub-region by the census population of bovines for each country within the sub-region, according to the following formula:

$$\begin{aligned} &census\ population \times [(sub\ regional\ mean\ live\ weight \\ &\times LSU_{calves} \times P.pop_{calves}) + (sub\ regional\ mean\ live\ weight \\ &\times LSU_{young\ 1-2yrs} \times P.pop_{young\ 1-2yrs}) \\ &+ (sub\ regional\ mean\ live\ weight \times LSU_{adults} \times P.pop_{adults})] \end{aligned}$$

Whereby,

$P.pop_{calves}$, $P.pop_{young\ 1-2years}$, $P.pop_{adults}$ represents, respectively, the proportion (P.pop) of calves, young (between 1 and 2 years of age) and adults in the total living cattle population, as calculated from Eurostat animal population data.

LSU_{calves}, $LSU_{young\ 1-2years}$, LSU_{adults} represents, respectively, the livestock unit ratios (LSU) for calves, young and adults as defined by Eurostat (15).

And, *sub regional mean live weight* represents the calculated mean live weight for adult cattle at the sub regional level.

Determination of the Mean Live Weight of Adult Cattle

The mean live weight of adult cattle is estimated by calculating a Generic mean live weight at slaughter from the production data which is then multiplied by a correction factor, derived from the Eurostat reference dataset.

Mean weight of live adult cattle = Generic mean live weight at slaughter * Correction factor (1.15).

Generic Mean Live Weight at Slaughter

The Generic mean live weight at slaughter, comprised of the weights of all the cattle slaughtered regardless of their production category, is calculated from annual production data (FAOSTAT), using the carcass to live weight conversion coefficient (÷ 0.54, formula 4.2.2.2), as defined by Eurostat:

$$\text{Generic mean live weight at slaughter (kg)} = \frac{\text{Generic carcass weight (kg)}}{\text{conversion coefficient (0.54)}}$$

Determination of the Correction Coefficient

Using reference datasets (Eurostat and several national detailed reports), where slaughter data are detailed by production category [adults, young (between 1 and 2 years of age), calves (<1 year of age)], it was estimated that, on average, the mean weight of live adult cattle was 15% higher than the Generic mean live weight at slaughter.

$$\frac{mean\ live\ weight\ of\ adults\ at\ time\ of\ slaughter}{Generic\ mean\ live\ weight\ at\ slaughter} = 1.15$$

Therefore, applying an add-on factor of 15% (×1.15) to the Generic mean live weight at slaughter is the best fit model to obtain the mean live weight of adult cattle when compared to the reference datasets (Eurostat and country specific data).

Sub-Regional Mean Live Weight

Countries were grouped by sub-region as defined by livestock unit classifications (13). A sub-regional mean live weight was then determined by calculating the average of the mean live weight of adult cattle for countries within the sub-regional grouping.

Swine biomass was calculated according to the following formula:

$$\begin{aligned} &(live\ weight \times number\ slaughtered) + (census\ population \\ &\times sow\ weight \times 0.09) \end{aligned}$$

Whereby,

live weight × *number slaughtered* represents the expected biomass of fattening pigs slaughtered in a country in 1 year,

And *census population* × *sow weight* × 0.09 represents the expected biomass of pigs retained for breeding purposes, calculated with the following considerations:

- The number of boars for breeding purposes is negligible compared to the number of sows;
- Sow weight: the standard weight of a sow in Europe is 240 kg (9). This weight was adapted by region using livestock unit ratios (Americas = 240 kg, Asia and the Pacific = 240 kg, Africa = 192 kg);
- 0.09 is the expected percentage of sows in a given swine population, as calculated from Eurostat animal population data.

Poultry biomass was calculated according to the following formula:

$$\begin{aligned} &(live\ weight\ chicken \times number\ of\ chicken\ slaughtered) \\ &+ (live\ weight\ turkey \times number\ of\ turkey\ slaughtered) \\ &+ (live\ weight\ ducks \times number\ of\ ducks\ slaughtered) \\ &+ (live\ weight\ geese \times number\ of\ geese\ slaughtered) \end{aligned}$$

Equidae biomass was calculated according to the following formula:

$$\begin{aligned} &(live\ weight\ horse \times horse\ census\ population) \\ &+ (live\ weight\ donkey \times donkey\ census\ population) \\ &+ (live\ weight\ mules \times mule\ census\ population) \end{aligned}$$

The live weight of horses, donkeys, and mules was calculated for regions where equine slaughter is common and data were

available. For regions where equine slaughter is not practiced and/or where data were unavailable, live weights were adapted using livestock unit ratios.

Sheep and goat biomass were calculated according to the following formula:

$$\left(live\ weight\ \times\ number\ slaughtered\right) + \left(census\ population - \frac{number\ slaughtered}{1.5}\right) \times\ 75\ kg$$

Whereby,

$\left(live\ weight\ \times\ number\ slaughtered\right)$ represents the expected biomass of sheep and goats slaughtered in a country in 1 year,

And $\left(census\ population - \frac{number\ slaughtered}{1.5}\right) \times 75\ kg$ represents the expected biomass of animals retained for breeding purposes, calculated with the following considerations:

- 1.5 is the average number of breeding cycles per year;
- The standard weight of a breeding small ruminant in Europe is 75 kg (9). This weight was used globally based on livestock unit ratios.

Rabbit biomass was calculated according to the following formula:

$$\left(live\ weight\ \times\ number\ slaughtered\right) + \left(census\ population - \frac{number\ slaughtered}{5}\right) \times\ 4.5\ kg$$

Whereby,

$\left(live\ weight\ \times\ number\ slaughtered\right)$ represents the expected biomass of rabbits slaughtered in a country in 1 year,

And $\left(census\ population - \frac{number\ slaughtered}{5}\right) \times 4.5\ kg$ represents the expected biomass of animals retained for breeding purposes, calculated with the following considerations:

- 5 is the average number of breeding cycles per year;
- The standard weight of a breeding doe is 4.5 kg.

Camelid and cervid biomass were calculated according to the following formula:

$$standard\ weight\ \times\ census\ population$$

According to the following considerations (16):

- Standard weight cervid: 80 kg
- Standard weight camel: 600 kg
- Standard weight, llama/alpaca: 100 kg

Farmed fish biomass was included in the total biomass only for countries that included aquaculture in their reported data on antimicrobials intended for use in animals. Aquaculture data are collected in WAHIS and FAOSTAT as tons produced annually, which were converted to kilograms for the animal biomass calculation.

Data on farmed crustaceans, molluscs and amphibians were excluded given the relatively small size of these populations, and inconsistency in their reporting.

Cats and dogs have not yet been included in the calculation of animal biomass due to inconsistency in reporting of their populations, and lack of information on average weights. For the countries where companion animal data was available, their contribution to overall animal biomass was found to be relatively minor (<1%). In the future, an analysis of companion animal data will hopefully become feasible.

DISCUSSION

Limitations

The OIE data collection on antimicrobials intended for use in animals is at an early stage of development and caution should therefore be taken when interpreting the data of the first years of data collection.

Multiple data sources are used by the different countries including imports, wholesalers data, marketing authorization holder declarations, and veterinary prescription. The level of accuracy of the data may be different according to the data source, for example import data may be relatively imprecise compared to marketing holder declarations or veterinary prescriptions.

In some cases, reporting of many different data sources can also result in over-estimated, duplicated or overlapping data. The OIE works with its Member Countries to correct thee issues wherever possible.

The OIE will continue to support its Member Countries through its Regional Trainings for National Focal Points for Veterinary Products, where the guidelines are reviewed and Member Countries can ask the OIE questions and share experiences with their peers.

As stated in the annual European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) report, 3–4 years are needed to establish a valid baseline for the data on sales of veterinary antimicrobial agents.

The animal biomass methodology was developed taking into account internationally available data. The level of detail of information available on a global level necessitated the development of a methodology partly based on informed assumptions or extrapolations, which cannot accurately represent the situation in every country. For example, the methodology for calculating an average animal weight from slaughter data, necessitates a conversion coefficient from carcass weight to live weight at time of slaughter. Presently, the European conversion coefficients were used for all the countries, but it is not currently known how well these apply to other countries that may have different slaughter practices, different breeds etc.

In the absence of global animal population data detailed by age and production, extrapolations were also calculated from European references. The extent to which these age class distributions of species apply to other countries is still undergoing a validation process.

The methodology for calculating biomass in several species is based on the mean standard weight of animals for breeding. An effort was undertaken to adapt these mean standard weights between regions using livestock units (13). A review of how well these standard weights depict the variability at a regional and national level has been initiated.

For cervids, camelids, and equines in some regions, data on breeding cycles were not collected at the time of reporting, nor was slaughter data. Therefore, this information was taken from literature where necessary, or extrapolated from regions where data was available (such as in the case of live weights of equines). The extent to which these literature and extrapolated weights and reproduction rates represent the true situation in any country is expected to vary.

Imported and exported animals are commonly subtracted and added, respectively, from animal populations when calculating animal biomass, as done in ESVAC and CIPARS. This is done so that only animals raised in the country, the time during which they would have been treated with antimicrobials, are considered. Currently, available data does not support incorporation of imported and exported animals. Their contribution to overall animal biomass was found to be relatively minor when calculated for certain countries where data was available.

In development of the current denominator methodology, it was decided at this time not to include companion animals in the calculation of animal biomass. Data on populations of cats and dogs are available in WAHIS, however, many countries do not report these figures, or report them inconsistently. Another consideration is the need to better understand whether reported cat and dog populations represent owned or stray animals, as this would affect the likelihood of their treatment with antimicrobials.

For the countries where cat and dog populations were available, it was seen that their contribution to overall biomass was minor (<1%). However, as some countries do include antimicrobials used in companion animals in their reported quantitative data, there is expected to be a small effect on results by excluding these species. As excluding them decreases this denominator, this effect, if any, would be a minor increase in antimicrobial quantities adjusted for animal biomass.

In the future, a goal would be to provide a separate analysis for antimicrobial agents used in companion animals, as more countries are able to report these population data, and distinguish antimicrobial quantities by animal group.

Prospects

The OIE will continue working closely with its Member Countries to support them in calculating kilograms of active ingredients of antimicrobials. An automated system for this calculation (conversion of antimicrobial active ingredients in veterinary medicines into kilograms) will be developed over time to assist Member Countries in this effort. This automated system will particularly help Member Countries with the burden of manually calculating kilograms of active ingredients and avoid errors with these calculations.

The OIE will also continue to refine its methodology for the calculation of animal biomass, based on globally available data, and communication with its Member Countries through its regional offices.

An important next step in this process is collaboration with the OIE World Animal Health Information and Analysis Department (WAHIAD). In consultation with the OIE *ad hoc* Group on Antimicrobial Resistance, new species and animal sub-categories have been added to the OIE World Animal Health Information System (WAHIS) data collection guidelines. These new population sub-categories are now being implemented in WAHIS and will allow to refine the data on animal biomass over time.

OIE-WAHIS, the next generation of the WAHIS data collection interface, is currently in development and will incorporate further updates to the collection of global animal population data. In addition to more sub-categories representing detailed production data when Member Countries are able to supply it, the interface will also include free text boxes allowing for description of the reported data. OIE-WAHIS will also additionally support the reporting of data on average live weights and number of animals slaughtered in Member Countries.

Aside from collection of more detailed global animal population data, more work is needed to validate some of the conversion coefficients, breeding cycles and population distribution ratios used in the methodology, which were extrapolated from European data as necessary. Particularly, better understanding potential regional variation in carcass conversion coefficients (for estimating live weights) and annual multiplication rates of species living <1 year (i.e., "cycle factor") are necessary within the current methodology to ensure its applicability on a global scale. The OIE is currently working with its Regional Offices to obtain better estimates on these variables across regions.

The third AMU report published by the OIE in 2019 clearly shows the significant commitment of OIE Member Countries to the development of data collection systems on antimicrobial agents intended for use in animals. The capacity to measure trends over time is progressing each year, and is critical to the international effort to promote the responsible and prudent use of antimicrobial agents in animals.

Simultaneously, as more precise data on animal populations becomes globally available, it is expected that the methodology for calculation of animal biomass will be further refined. With the concurrent development of quantitative data collection and calculation of animal biomass, this annual report will allow for increasingly effective comparisons on a global and regional scale.

DATA AVAILABILITY

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Calculation of Antimicrobial Use Indicators in Beef Feedlots—Effects of Choice of Metric and Standardized Values

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The potential for antimicrobial use (AMU) to lead to the development of antimicrobial resistant bacteria is an increasingly important priority in human and veterinary medicine. Accurate AMU quantification is essential to assessing the risk of antimicrobial resistance due to AMU. The quantification of AMU in production animals can be difficult, and feedlot beef cattle present a number of unique challenges. This paper presents selected parenteral data from western Canadian beef feedlots to illustrate variations in interpretation of AMU that can arise from the use of different metrics and standards. Specific examples presented compare the number of animal daily doses calculated from a given amount of antimicrobial drug (AMD) using actual and estimated weights of cattle at exposure, dose-based to weight-based indicators representing the same amount of antimicrobial, dose-based AMU indicators using different estimated durations of effect (DOE), and AMU indicators calculated using different standard weights of cattle at exposure. Changing these factors when calculating AMU indicators can have notable influences on the results obtained. Transparency about the methods used to calculate AMU indicators is critical to ensure that comparisons of use among different populations is meaningful and accurate.

Keywords: cattle, animal daily dose, quantification, comparison, duration of effect

INTRODUCTION

The potential for antimicrobial use (AMU) to promote selection of antimicrobial resistant bacteria is a subject of increasing priority to stakeholders in public and animal health, policy making and international trade (1). In particular, AMU in food-producing animals is under intensifying scrutiny because of potential public health risks putatively associated with contamination of the environment and food products with resistant bacteria (2–4) and direct transmission of resistant bacteria (5). However, the collection and analysis of AMU data in production livestock can be logistically challenging for a number of reasons, and these difficulties have been repeatedly identified as a barrier to understanding AMU and resistance in this context

(6, 7). Nonetheless, information about AMU in food-producing animals is critically important for assessing relationships between AMU and antimicrobial resistance (AMR), to understand variability of AMU among different populations, to design or improve AMU monitoring systems, and to inform antimicrobial stewardship efforts.

Antimicrobial drugs are regularly used in North America to maintain feedlot cattle health (8). Almost 90% of feedlots with more than 1,000 head of cattle in the United States reported administering AMDs to cattle by injection or in feed or water in a survey administered in 2016 (9). Bovine respiratory disease (10) and control of liver abscesses (11) are two common therapeutic indications for AMU in feedlot cattle. Use of antimicrobials for production purposes (e.g., growth promotion and feed efficiency) is now limited in Canada and the United States to non-medically important antimicrobials, such as ionophores, and is restricted in Mexico (12–14).

Attempting to quantify AMU in beef cattle is accompanied by many unique challenges in comparison with other production animals. While other species, such as poultry, have a consistent and short production cycle, the life-span, and the related length of time that cattle are intensively managed for finishing is relatively long and can vary significantly. Most cattle raised for beef production in North America are harvested when live bodyweights range from 500 to 640 kg (about 1,100–1,400 lb), and when cattle are typically <2 years of age. The feeding period (the time spent in a feedlot or intensive finishing operation) of beef cattle can vary depending upon weight at placement, feeding conditions, and whether a leaner or fatter animal at finish is desired, but is generally from 90 to 300 days (15). Because of the lengthy feeding period and the relatively large size of cattle at harvest, differences in weight gained during the feeding period can vary by several hundred kilograms. Due to common feedlot practices wherein animals are re-sorted with some frequency to maintain pens of uniformly sized cattle to facilitate feeding and marketing, it can also be difficult to follow individual animals and pen groups because the animal units that make up the pens can change. Finally, while not a problem unique to estimating AMU in feedlot cattle, the duration of effect (DOE) of some antimicrobial drugs (AMD) has not been internationally established (31).

There are many approaches to the quantification of AMU, each with their own unique advantages and disadvantages; no single method is considered to be ideal in all circumstances (16). Measurements used to quantify AMU typically include a numerator describing the amount of AMD animals received and a denominator intended to normalize the numerator by animal weight or the at-risk number of animals or animal-days (7). Taken together, the numerator and denominator are defined as "indicators" of AMU (17). Mass of active ingredient (mg or kg) is intuitive and easily understood as a numerator, especially by lay people. However, this type of measurement can be misleading and inaccurate because it does not account for variations in the mg/kg dosage of antimicrobials (18). Accordingly, dose-based metrics have been adopted by many research groups (19, 20). Dose-based metrics have the advantage of accounting for differences among drugs in concentration and DOE, thus providing an AMU measurement with a more realistic interpretation of the relative contribution of different antimicrobials than weight or count-based metrics (18). In this system, a defined daily dose (average of the range of dosages in units of mg/kg animal/day) must be described for the population of interest for each drug, age-group, and species (21). Employing the defined daily dose and a standard animal weight at exposure, the number of animal daily doses of a particular AMD contained in a given amount of that AMD can be estimated. The selected standard weight selected can have a significant effect on calculated dose-based metrics and should be clearly stated for optimal data interpretation. The standard weight is at best always an approximation, but knowledge of the weight used makes it possible to recalculate metrics for different purposes using other potentially relevant weights (18).

It should be noted that confusion can arise in dose-based data interpretation because terminology has not been uniformly applied throughout the literature and with different methodologies; it is important to recognize these discrepancies when assessing published AMU data and calculations. The World Health Organization[1] defines the defined daily dose (DDD) in people as "the assumed average maintenance dose per day for a drug used for its main indication in adults;" the DDD as applied in human medicine therefore has mg/day units. In contrast, the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) group uses the terminology DDD_{vet} to refer to the mg/kg/day dosages for AMDs in different species; it is recommended that the specific term DDD_{vet} be reserved for ESVAC use to avoid confusion (22). Similarly, terminology like "animal daily dose" (ADD) has been used in the literature both to refer to the mg/kg/day dosage (23, 24) and mg/day dosage for an animal of standard weight (18, 25). Since there is such a range in terminology, it is important to clearly state the units associated with each metric reported (26).

Selection of an appropriate denominator to quantify the population of at-risk animals is critical to data interpretation, especially when disparate animal populations are compared. For example, if AMU in mg on a poultry flock was compared to use in mg in a herd of the same number of cattle without normalization, it would seem that the antimicrobial amounts used in the cattle were relatively high compared to the poultry if the larger size of cattle (necessitating a larger dose of AMD per animal) was not considered. For description of AMU at the farm level, ESVAC suggests as a denominator the number of animals present "in a certain weight group or production type and the time present" (27). Dose-based numerators are frequently reported with the denominator of number of exposed animals of a standard weight (28, 29). Number of animal-days has also been advocated as a denominator (17). Denmark reports AMU in different species production classes as the estimated treatment proportion, or the number of defined animal daily doses for each antimicrobial agent by species (mg/kg/day) divided by 1,000 standard animals per day (30).

[1]World Health Organization. *WHO Collaborating Centre for Drug Statistics Methodology*. World Health Organization. Available online at: https://www.whocc.no/ (accessed April 15, 2018).

For national reports of antimicrobial sales data, the total body weight of the animal population is recommended by ESVAC as a denominator (27). The population correction unit (PCU), as defined by ESVAC, is an example of a theoretical estimate of exposed biomass. It is calculated by multiplying the number of animals slaughtered during a given period of time by the standard weight of the animals at the time of exposure (31); the national PCU is then obtained by summing PCU from all sectors of food animal production. The PCU term can be useful as a denominator because 1 PCU is equal to 1 kg of any category of exposed animal and is thus interchangeable among different species, and the overall mg (AMD) per PCU indicator is frequently employed in national reports of veterinary antimicrobial sales data in European countries (19, 32). The Public Health Agency Canada (PHAC) and the U.S. Food and Drug Administration (FDA) are both currently evaluating methods to calculate accurate biomass denominators in their respective countries. Standard weights in use by ESVAC are currently employed by PHAC for calculations of PCU until Canadian estimates can be determined (33). The FDA has recently proposed calculation of a biomass denominator for each of the four major food producing species in the U.S. (cattle, swine, chickens, and turkeys), which would be referred to as a target animal biomass (TAB). The TAB would be calculated by multiplying the estimated number of animals in each group in the U.S. by the average weight at slaughter in kg. Antimicrobial sales data would be reported as the sum in mg of all AMDs for a given target animal species, divided by the species-specific TAB (34). The choice of the standard weight used, as well as adjustments made for animal lifespan, can markedly affect PCU calculations (35), and estimates used for these computations should be clearly stated.

The aim of this publication is to present selected parenteral data from a study of AMU in 36 beef feedlots in western Canada (36) to demonstrate the influence of factors such as cattle weight estimates, choice of indicator, and estimates of DOE of AMD on the calculations of AMU indicators. Four examples will be explored: (1) comparison of dose-based AMU metrics (numerator only) using actual and estimated weights of cattle at exposure; (2) comparison of dose-based to weight-based (e.g., mg of AMD) AMU indicators; (3) comparison of dose-based AMU indicators using different estimated DOE; and (4) comparison of AMU indicators calculated using different standard weights.

MATERIALS AND METHODS

The AMU data used for examples in this publication were collected from mixed-breed cattle (n = 2,615,082) arriving onto 36 western Canadian feedlots from November 1, 2008 to October 31, 2012, and are comprehensively summarized with descriptive statistics in a separate report (36). Briefly, information about individually administered (parenteral and bolus-dosed) and in-feed AMU for therapeutic and production purposes was recorded from placement until animal exit (death or shipping for slaughter). Data collected for individually administered AMU included unique identification number of the exposed animal, date, animal weight at time of administration, active AMD ingredient, dosage, route, reason for administration (metaphylactic or treatment), and disease/syndrome. Data collected for in-feed AMU was less comprehensive and included the production lot of the exposed cattle, feed delivery date, number of animals in the production lot each day, and number of animals receiving each type of in-feed AMD. Based on their date of arrival into the feedlot, the cattle were divided into 4 placement cohorts (PC1, PC2, PC3, and PC4). Cattle arriving between November 1, 2008 and October 31, 2009 comprised placement cohort 1 (PC1), PC2 comprised cattle arriving between November 1, 2009 and October 31, 2010, PC3 comprised cattle arriving between November 1, 2010 and October 31, 2011, and PC4 comprised cattle arriving between November 1, 2011 and October 31, 2012. While cattle were owned and managed by multiple individuals and companies, their healthcare was overseen by a single veterinary practice (Feedlot Health Management Services Ltd, Okotoks, Alberta; Feedlot Health). Data were summarized and metrics/indicators calculated using SAS® software (Windows version 9.4, SAS Institute, Cary, North Carolina).

For the purposes of this study, the resolution of the parenteral data was superior to the in-feed data collected in that individual animal identification, actual dose of AMD used, and the weight of animal at exposure (in nearly all cases) were recorded for parenteral data and not in-feed data. Therefore, to clearly illustrate the influence of changing various factors on AMU calculations using the same comprehensive data, comparative analyses for AMU of 3 parenteral AMD (tetracyclines, macrolides, and beta-lactams) will be used for all examples, although the principles described are generally applicable to other parenteral AMD and in-feed AMD. These 3 AMD also tend to be administered at different times in the feeding period which makes them particularly useful for contrast in examples where weight at exposure is important.

In this study, "animal daily dose" (ADD) will be employed as the dose-based AMU metric (18); ADD will refer to the mg/day dosage for an animal of standard weight and ADD_{kg} will designate the mg/kg/day dosage. The number of ADD (nADD) in a given amount of antimicrobial will be calculated using [Equation 1; (25)]. Depending upon the purpose of the calculation, the actual weight of the animal at exposure in kg or a standard weight will be used.

$$nADD = \frac{Qty\ of\ active\ substance\ in\ mg\ administered}{ADD\ (mg\ per\ kg\ per\ day) * weight\ \left(kg\right)\ of\ animal} \quad (1)$$

Denominators presented in the examples will include "per 100 cattle-at-risk" and "per kg biomass." The denominator includes the entire time that an animal is at risk for antimicrobial exposure from placement at the feedlot until exit; in other words, nADD/100 cattle-at-risk indicates the number of daily doses of antimicrobial applied on average to 100 cattle in the population from placement to exit. The kg biomass denominator will be calculated as described for PCU, but PCU-specific terminology will

not be used because only 1 species of animal is being described (24):

$$kg\ biomass = Number\ of\ animals * standard\ weight\ (kg) \quad (2)$$

RESULTS

Example 1: Comparison of Calculation of Dose-Based Metric of AMU (Numerator) Using Actual and Estimated Weights of Cattle at Exposure

To determine the effect of the weight of the animal on the calculation of dose-based AMU metrics, the nADD calculated with actual exposed animal weights was compared with the nADD calculated from the same dataset using a uniform standard weight. The daily doses (ADD_{kg}) in mg/kg/day in the surveilled feedlots for each parenteral drug to which cattle were exposed were calculated by dividing the administered dose (mg/kg) by the estimated exposure days represented by one standard treatment, or the DOE (16). For simplicity, only use of parenteral macrolides, tetracyclines, and beta-lactams are presented (**Table 1**).

In this dataset, cattle with recorded individual weights were treated with the parenteral antimicrobials listed in **Table 1** 2,196,473 times. An ADD was calculated for each antimicrobial administration by multiplying the ADD_{kg} for the antimicrobial by the actual exposure weight (kg) of the treated animal. The recorded mg administered to the animal was then divided by the calculated ADD to yield the nADD. Examples of calculations for three observations are presented in **Supplementary Table 1**. These calculations were performed individually for each observation and then the nADD were summed by antimicrobial type and divided by the number of actual animals exposed to yield the mean nADD at each administration (**Table 2**). As would be expected, the mean of the nADD for each administration approximated the DOE for each antimicrobial in days. The nADD for macrolides, tetracyclines, and beta-lactams for the same dataset were then estimated using a standard weight (the mean weight of cattle at time of exposure to any AMD) to calculate the ADD rather than a known weight. The mean cattle weight at exposure to AMDs in this dataset (tylosin administered as part of hormone implants excluded) was 336 kg (standard deviation 98 kg; range 45–909 kg). Calculation of ADD for each antimicrobial type was performed based upon the mean weight estimate of 336 kg at the time of antimicrobial exposure and then used to estimate the nADD comprising the mg of antimicrobials used in the population (**Table 3**). The ADD_{kg} was multiplied by the mean weight to generate the standard animal daily dose (ADD) for each drug. The total mg of antimicrobial used in the population was divided by the standard ADD to yield the nADD. Variation between the estimated nADD using the mean weight at exposure and the nADD calculated using actual weight at exposure is shown as a percentage change between the two ([estimated nADD – "actual" nADD]/"actual" nADD).

The use of estimated vs. body weights measured at the time of drug administration influenced the results of the nADD calculation for most AMDs in the analysis, with the overall

TABLE 1 | Administered dose, estimated duration of effect (DOE), and the calculated animal daily dose in mg/kg/day (ADD_{kg}) of selected antimicrobial drugs used parenterally throughout the study.

Parenteral antimicrobial drug	Administered dose (mg/kg)	DOE (days)	ADD_{kg} (mg/kg/day)
Macrolides			
Tulathromycin	2.5	3	0.83
Tilmicosin	10.0	3	3.33
Gamithromycin	6.0	3	2.00
Tildipirosin	4.0	3	1.33
Tetracyclines			
Oxytetracycline (100 mg/ml)	6.7	1	6.70
Oxytetracycline (200 mg/ml)	20.0	2	10.00
Oxytetracycline (300 mg/ml)	30.0	3	10.00
Beta-lactams			
Ceftiofur hydrochloride or sodium	1.1	1	1.10
Ceftiofur crystalline free acid	6.6	3	2.20
Procaine penicillin	20.0	3	6.67

TABLE 2 | Number of animal daily doses (nADD) of parenteral antimicrobial drugs based on actual recorded animal weights and actual number of animals exposed.

Parenteral antimicrobial drug	Number of administrations	Sum of nADD	Mean of nADD of each administration	Standard deviation of mean of nADD
Macrolides				
Tulathromycin	620,058	1,869,247	3.01	0.22
Tilmicosin	68,087	214,741	3.15	0.22
Gamithromycin	9,260	28,274	3.05	0.24
Tildipirosin	3,358	9,195	2.74	0.14
All macrolides	700,763	2,121,457		
Tetracyclines				
Oxytetracycline (100 mg/ml)	4,321	4,375	1.01	0.05
Oxytetracycline (200 mg/ml)	952,951	1,899,370	1.99	0.11
Oxytetracycline (300 mg/ml)	387,256	1,169,307	3.02	0.23
All tetracyclines	1,344,528	3,073,052		
Beta-lactams				
Ceftiofur hydrochloride or sodium	203,671	213,103	1.05	0.05
Ceftiofur crystalline free acid	2,440	7,589	3.11	0.13
Procaine penicillin	520	1,649	3.17	1.16
All beta-lactams	206,631	222,341		
Sum of antimicrobials	2,251,922	5,416,850		

TABLE 3 | Calculation of number of animal daily doses (nADD) using mean weight at exposure for the entire population compared to calculation of nADD using actual weight at exposure (from **Table 2**) for different antimicrobial types.

Parenteral antimicrobial drug	ADD_{kg} (mg/kg/day)	Mean weight (kg)	Standard ADD (mg/day)	Antimicrobial used (mg)	nADD (mean weight)	nADD (actual weight)	Variation (%)
Macrolides							
Tulathromycin	0.83	336	280.0	400,310,350	1,429,680	1,869,247	−23.5
Tilmicosin	3.33	336	1,120.0	189,139,740	168,875	214,741	−21.4
Gamithromycin	2.00	336	672.0	15,009,300	22,335	28,274	−21.0
Tildipirosin	1.33	336	448.0	3,578,760	7,988	9,195	−13.1
All macrolides					1,628,878	2,121,457	−23.2
Tetracyclines							
Oxytetracycline (100 mg/ml)	6.70	336	2,251.2	9,386,200	4,169	4,375	−4.7
Oxytetracycline (200 mg/ml)	10.00	336	3,360.0	6,882,331,720	2,048,313	1,899,370	7.8
Oxytetracycline (300 mg/ml)	10.00	336	3,360.0	3,435,537,840	1,022,482	1,169,307	−12.6
All tetracyclines					3,074,964	3,073,052	0.0
Beta-lactams							
Ceftiofur hydrochloride or sodium	1.10	336	369.6	113,460,385	306,982	213,103	44.1
Ceftiofur crystalline free acid	2.20	336	739.2	6,793,000	9,190	7,589	21.1
Procaine penicillin	6.67	336	2,240.0	4,334,700	1,935	1,649	17.4
All beta-lactams					318,107	222,341	43.1
All antimicrobial drugs					5,051,948	5,416,850	−7.3%

nADD underestimated by 7.3% when mean weights were used for the calculation. Even greater discrepancies were noted when individual antimicrobial classes were examined. For example, macrolide use was underestimated by 23.2% and beta-lactam use was overestimated by 43.1% when mean weights rather than actual weights were used. To explore this contrast further, the mean weights at time of exposure (specifically for macrolides and beta-lactams) were determined (**Supplementary Figure 1**). The mean exposure weight for macrolides was 267 kg and for beta-lactams was 484 kg.

To demonstrate the effect of accurately estimated weights on calculation of nADD, the mean exposure weights (for macrolides and beta-lactam) were used to recalculate nADD in **Table 4**; similar to **Table 3**. Overall, the variation between the two calculations was markedly decreased by using more specific weights for the antimicrobial classes.

Example 2: Comparison of Dose-Based and Weight-Based AMU Indicators

Part 1: Comparison of Use of Two Different Antimicrobial Classes

To directly compare AMU quantification in different AMD classes (dose-based vs. weight-based indicators), parenteral AMU data for macrolides and tetracyclines from two feedlots (A and B) are presented. These particular feedlots and antimicrobials were compared because of the marked contrast in the proportion of cattle exposed to the two antimicrobial types in the two feedlots, and the relatively lower mg/kg dosage of macrolides; accentuating differences between dose-based and weight-based metrics. As can be seen in **Figure 1**, the proportion of cattle exposed to tetracyclines parenterally was higher in Feedlot B than A, and the proportion of cattle exposed to macrolides was higher in Feedlot A than B.

The dose-based AMU indicator selected for this example was the nADD per 100 cattle-at-risk. Prerequisite for this calculation was the ADD in mg/kg/day or ADD_{kg} (calculated in **Table 1**). The ADD [total mg of the particular antimicrobial drug administered to a "standard" animal (mg/day)], was then obtained by multiplying the ADD_{kg} (mg/kg/day) by the mean cattle weight at exposure averaged over both feedlots (kg) (**Table 5**).

The total mg of macrolides and tetracyclines used in each feedlot were summed from administration records. The total mg amount of each AMD type was then divided by the specific ADD for each drug to yield the nADD consumed in each feedlot, and the summed total mg and the nADD were divided by the number of cattle-at-risk/100 to provide the mg/100 cattle-at-risk and the nADD/100 cattle-at-risk (**Table 6**). **Figure 2** presents AMU of both AMD types, depending upon whether mg AMD or nADD are used in the calculation.

It can clearly be seen that mg as a measurement of AMU confuses interpretation when different classes of AMD are used at disparate levels in the two populations compared. Macrolide use was more common in Feedlot A, while Feedlot B used proportionately more tetracyclines. Since tetracycline mg/kg dosages tend to be higher than macrolides, this inflated the AMU measurement in Feedlot B compared to Feedlot A when the mg metric was used. When the nADD metric (accounting for differences in concentration between the two AMD classes) was used, AMU measurements between the two feedlots were much closer.

TABLE 4 | Use of mean weight at exposure for macrolides and beta-lactams compared to actual weight at exposure to calculate the number of animal daily doses (nADD) of the 2 antimicrobial classes.

Parenteral antimicrobial drug	ADD_{kg} (mg/kg/day)	Mean weight (kg)	Standard ADD (mg/day)	Antimicrobial used (mg)	nADD (mean weight)	nADD (actual weight)	Variation (%)
Macrolides							
Tulathromycin	0.83	267	223	400,310,350	1,799,148	1,869,247	−3.8
Tilmicosin	3.33	267	890	189,139,740	212,517	214,741	−1.0
Gamithromycin	2.00	267	534	15,009,300	28,107	28,274	−0.6
Tildipirosin	1.33	267	356	3,578,760	10,053	9,195	9.3
Overall macrolides					2,049,825	2,121,457	−3.4
Beta-lactams							
Ceftiofur hydrochloride or sodium	1.10	484	532.4	113,460,385	213,111	213,103	0.0
Ceftiofur crystalline free acid	2.20	484	1,065	6,793,000	6,378	7,589	−15.9
Procaine penicillin	6.67	484	3,227	4,334,700	1,343	1,649	−18.6
Overall beta-lactams					220,832	222,341	−0.68

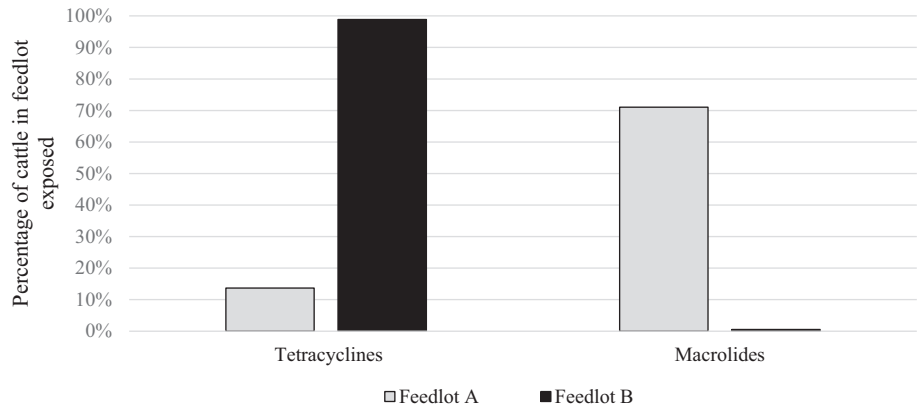

FIGURE 1 | Percentage of cattle exposed to tetracyclines and macrolides parenterally in Feedlots A and B.

TABLE 5 | Parenteral macrolides and tetracyclines used in Feedlots A and B and the calculation of the animal daily dose (ADD).

Parenteral antimicrobial drug	ADD_{kg} (mg/kg/day)	Mean weight (kg)	ADD (mg/day)
Macrolides			
Tulathromycin	0.8	375.6	300.5
Tilmicosin	3.3	375.6	1239.5
Tetracyclines			
Oxytetracycline (100 mg/ml)	6.7	375.6	2516.5
Oxytetracycline (200 mg/ml)	10.0	375.6	3756.0
Oxytetracycline (300 mg/ml)	10.0	375.6	3756.0

Part 2: Intra-Class Comparison of Antimicrobial Drug Use

To directly compare AMU quantification of different types of the same drug class obtained by dose-based vs. weight-based indicators, macrolide use in Feedlot A from two groups of cattle was considered: Cattle entering Feedlot A between November 1, 2008 and October 31, 2009 comprised Placement Cohort 1, and cattle entering Feedlot A between November 1, 2010 and October 31, 2011 comprised Placement Cohort 3. The relative frequency of tilmicosin use decreased while the relative frequency of tulathromycin use increased over time (**Figure 3**). Use of parenteral macrolides (either tulathromycin 2.5 mg/kg or tilmicosin 10 mg/kg) expressed as mg/100 cattle-at-risk and nADD/100 cattle-at-risk is presented; overall and by type (**Table 7**, **Figure 4**). The average cattle weight in Feedlot A (338 kg) and the number of cattle in each placement cohort was used to calculate the kg biomass for each placement cohort.

Because of the substitution of tulathromycin (lower mg/kg dose) for tilmicosin (higher mg/kg dose) that occurred in Feedlot A over time, macrolide use appears to decrease when the mg/100 cattle-at-risk indicator is used. However, if calculated as nADD/100 cattle-at-risk it can be seen that macrolide use in this feedlot was relatively stable or only slightly decreased over time.

TABLE 6 | Calculation of total mg antimicrobial drug/100 cattle-at-risk and number of animal daily doses (nADD)/100 cattle-at-risk for parenteral macrolides and tetracyclines in Feedlots A and B.

	Total mg	ADD (mg/day)	nADD	Number of cattle	Number of cattle/100	mg/100 cattle-at-risk	nADD/100 cattle-at-risk
FEEDLOT A							
Macrolides							
Tulathromycin	36,059,760	300	120,007	103,272	1,033	34,917	116
Tilmicosin	73,202,700	1,239	59,059	103,272	1,033	70,883	57
Tetracyclines							
Oxytetracycline (100 mg/ml)	107,600	2,517	43	103,272	1,033	104	0
Oxytetracycline (200 mg/ml)	43,719,800	3,756	11,640	103,272	1,033	42,335	11
Oxytetracycline (300 mg/ml)	80,155,500	3,756	21,341	103,272	1,033	77,616	21
Macrolides and tetracyclines	233,245,360		212,090	103,272	1,033	225,855	205
FEEDLOT B							
Macrolides							
Tulathromycin	2,710,190	300	9,020	582,133	5,821	466	2
Tilmicosin	0	1,239	0	582,133	5,821	0	0
Tetracyclines							
Oxytetracycline (100 mg/ml)	950,000	2,517	378	582,133	5,821	163	0
Oxytetracycline (200 mg/ml)	4,105,345,220	3,756	1,093,010	582,133	5,821	705,225	188
Oxytetracycline (300 mg/ml)	865,935,240	3,756	230,547	582,133	5,821	148,752	40
Macrolides and tetracyclines	4,974,940,650		1,332,954	582,133	5,821	854,606	230

Example 3: Comparison of Dose-Based Indicators Calculated Using Different Duration of Effect Estimates

As has been seen in the previous examples, the ADD_{kg} for each drug is assigned by dividing the administered dose by "the number of days of duration of the therapeutic effect of the substance (22)." However, the length of time an antimicrobial may exert selective pressure on bacteria is not always clear. For tulathromycin, a long acting macrolide frequently administered metaphylactically for BRD, many possible DOE could be proposed based on pharmacokinetic data and expert opinion. Three possibilities are given here: (1) Three days [plasma elimination half-life of the drug (37) and also a standard post-metaphylaxis interval, i.e., the number of days that must elapse before a metaphylactically exposed animal should be treated for BRD] (38). (2) Eight days (elimination half-life in the lung) (37) and value used in daily dose calculation for tulathromycin by ESVAC (22). (3) Fourteen days (estimated DOE in product literature from Zoetis) (39). For illustration purposes, the effect of these 3 different DOE on the nADD/100 cattle-at-risk calculation for one of the feedlots (Feedlot C) is shown (**Table 8**, **Figure 5**). Feedlot C contained 178,089 cattle over the course of the study with a mean weight of 291 kg at exposure to tulathromycin.

It can be seen from these data that as the DOE increases, the computed AMU indicator (nADD/100 cattle-at-risk) increased if the number of cattle-at-risk, the mg tulathromycin applied to the population, and the standard cattle weight were held constant. A DOE of 14 days more than quadrupled the calculated indicator of tulathromycin use in this feedlot compared to a DOE of 3 days, demonstrating the importance of DOE choice in the determination of AMU indicators.

Example 4: Comparison of Weight-Based AMU Indicators Calculated Using Different Biomass at Risk Estimates

To assess the effect of standard weights on AMU indicators using kg biomass as the denominator, 3 different standard weights were applied to data from Feedlot C (from the previous example). Since mg/kg biomass is a commonly reported indicator, mg tulathromycin is presented. Because only one type of AMD is measured by this indicator, no distortion of the calculated values is created by varying AMD concentration and DOE. The standard weights chosen were the known mean exposure weight for tulathromycin (291 kg in Feedlot C), the ESVAC standard heifer weight (200 kg), and the ESVAC standard steer and bullock weight (425 kg) (40). The kg biomass was calculated as previously described by multiplying the number of cattle-at-risk by the standard weight at time of exposure (**Supplementary Table 2**, **Figure 6**).

In contrast to DOE, as standard weight increased, the computed AMU indicator (mg/kg biomass) decreased with mg tulathromycin and the number of animals-at-risk held constant. A standard weight of 200 kg approximately doubled the calculated tulathromycin use indicator compared to a standard weight of 425 kg.

DISCUSSION

The presented examples show that variations in the animal weight or antimicrobial DOE can potentially have a profound effect on calculated AMU indicators. Standardization of measurement is critical whether AMU data will be used for temporal comparisons over time in the same population,

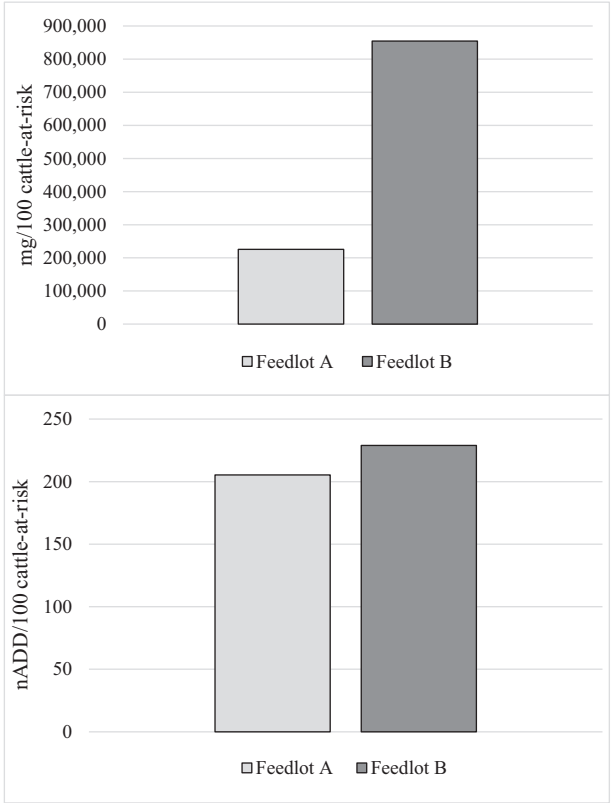

FIGURE 2 | Summed use of parenteral macrolides and tetracyclines in Feedlots A and B in mg antimicrobial drug/100 cattle-at-risk and number of animal daily doses (nADD)/100 cattle-at-risk.

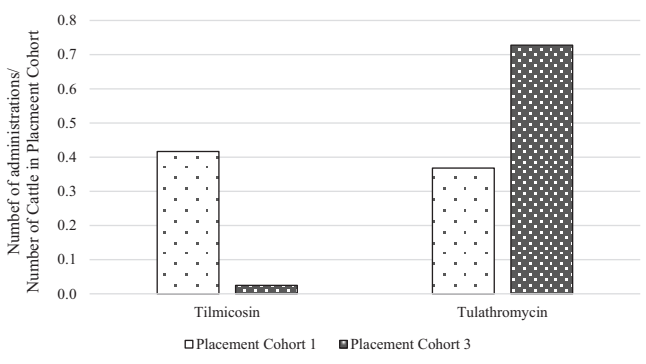

FIGURE 3 | Relative frequency of tilmicosin and tulathromycin use in placement cohorts 1 and 3* of Feedlot A. *Placement cohort 1 comprised cattle placed in feedlot between November 1, 2008 and October 31, 2009, and placement cohort 3 comprised cattle placed in feedlot between November 1, 2010 and October 31, 2011.

benchmarking, or estimation of selective pressure on development of bacterial resistance to antimicrobials (7). The four examples presented in this study each illustrate the influences of particular factors on the calculation of AMU indicators. Example 1 demonstrates that if dose-based metrics such as nADD are to be calculated, it is preferable to use standard weights specific to antimicrobial type for the most accurate results in cases where antimicrobials tend to be administered only at a specific point in the feeding period. Corresponding with the fact that parenteral macrolides are typically administered near the time of arrival at the feedlot, while beta-lactams are administered later in the feeding period, the mean exposure weight for macrolides was much lower than that of beta-lactams. While this type of adjustment has not been routinely done in the calculations of dose-based metrics for production animals such as swine and poultry that have smaller weight increases through the production cycle, it may be particularly important in beef cattle given the large variations in weight at exposure for drugs routinely given at placement vs. drugs given later in the feeding period. Because it is rare to have such detailed information, such as weights of cattle at exposure, as was available in this study, the use of standard weights specific to antimicrobial type may not always be feasible. However, the potential inaccuracy introduced by the use of one standard weight for all antimicrobials should be recognized. Example 2 demonstrated the advantages of dose-based metrics rather than weight-based metrics if there is variation in the AMD type used by populations that are to be compared. Weight-based metrics, such as mg or kg of active ingredient, are meaningless if AMD with different concentrations and DOE are being compared. In some food animal production systems with minimal variation in AMD type, this may not be as crucial, but in beef cattle, the routine use of both tetracyclines and macrolides, which have significant differences in mg/kg dosage, in herds makes this distinction particularly significant if comparing AMU among herds with disparate prescription practices. Employing weight-based metrics, herds administering more parenteral tetracyclines over macrolides would appear to have heavier AMU than herds administering more parenteral macrolides over tetracyclines, whereas dose-based metrics would tend to indicate the reverse. Consequently, emphasis on weight-based metrics and mg/kg reduction targets could even promote the use of macrolides in preference to tetracyclines, inadvertently encouraging the use of AMD of higher medical importance to humans (41). Comparing national sales data in mg/kg to animal census AMU data available in Denmark and the Netherlands, Bondt et al. (42) concluded that "simple country comparisons, based on total sales figures, entail the risk of serious misinterpretations, especially if expressed in mg per kg." They noted that to make meaningful international comparisons, the average dosage of the AMD used as well as relative differences in production animal species needed to be taken into account. However, at the national level, the collection of data with enough detail to calculate dose-based metrics is costly and time-consuming, leading most countries to employ the use of aggregated AMD sales data in mg as a proxy for AMU due to resource limitations (43). Recording of more detailed data about AMU by class and species-specific AMD dosages and applications on sentinel farms is recommended when feasible to complement sales data (44). There is ultimately no single AMU metric that is ideal in all situations and a balance must be struck between practicality, accuracy, efficiency, and clarity (16).

TABLE 7 | Calculation of total mg antimicrobial drug/100 cattle-at-risk and number of animal daily doses (nADD)/100 cattle-at-risk for macrolides in placement cohorts 1 and 3* of Feedlot A.

	Total mg	Mean weight (kg)	ADD_{kg} (mg/kg/day)	ADD (mg/day)	nADD	Number of cattle	Number of cattle/100	mg/100 cattle	nADD/100 cattle
Placement cohort 1									
Tilmicosin	38,283,600	338	3.3	1,127	33,980	28,200	282	135,757	120
Tulathromycin	6,911,910	338	0.8	282	24,539	28,200	282	24,510	87
All macrolides	45,195,510	338			58,519	28,200	282	160,267	207
Placement cohort 3									
Tilmicosin	2,189,100	338	3.3	1,127	1,943	30,339	303	7,215	6
Tulathromycin	14,935,620	338	0.8	282	53,026	30,339	303	49,229	175
All macrolides	17,124,720	338			54,969	30,339	303	56,444	181

**Placement cohort 1 comprises cattle placed in feedlot between November 1, 2008 and October 31, 2009, and placement cohort 3 comprises cattle placed in feedlot between November 1, 2010 and October 31, 2011.*

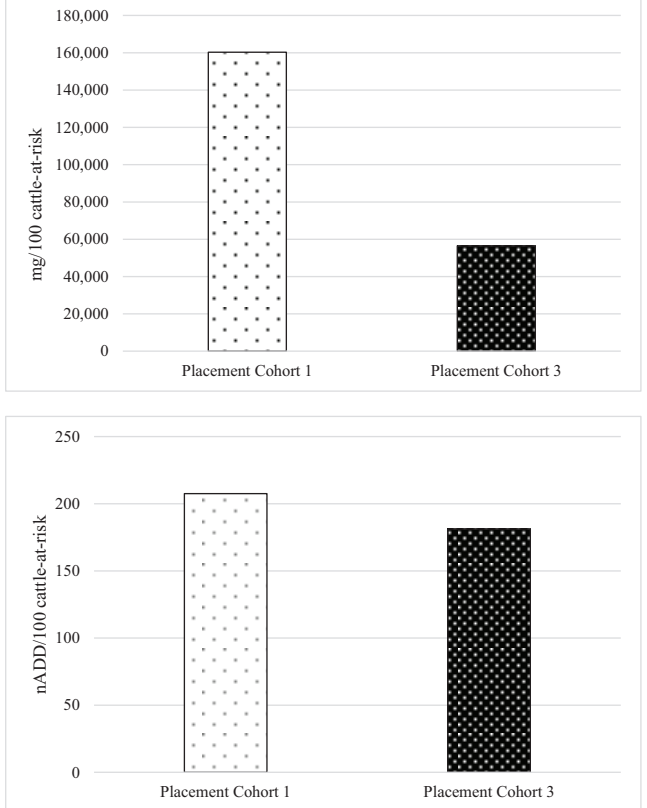

FIGURE 4 | Overall use of macrolides in Feedlot A in mg antimicrobial drug/100 cattle-at-risk and number of animal daily doses (nADD)/100 cattle-at-risk in placement cohorts 1 and 3*. *Placement cohort 1 comprises cattle placed in feedlot between November 1, 2008 and October 31, 2009, and placement cohort 3 comprises cattle placed in feedlot between November 1, 2010 and October 31, 2011.

For longer-acting antimicrobials such as macrolides, Example 3 illustrated that the choice of DOE in calculating AMU indicators was critical; use of longer DOE resulted in the calculation of higher dose-based use metrics. If benchmarking comparisons are performed, the actual value of the DOE is less important than ensuring that the same one is used for both populations. However, for studies quantifying AMU for the purposes of evaluating influence on bacterial AMR selection, the DOE choice will affect the AMR pressure intensity assigned to an AMD, but data to provide guidance on the correct DOE choice are often lacking. The concentration and persistence of macrolides in lung tissue for up to 14 days may be very important to selection of AMR in *Mannheimia haemolytica* (a bacteria related to BRD often present in the lungs of affected cattle). However, macrolides may not have as prolonged an effect on fecal bacteria such as *Enterococcus* spp. and *Escherichia coli*, bacteria frequently of interest in AMU/AMR studies, so it may not be appropriate to use the same DOE for considering AMR in all bacteria. More data are needed about the DOE of long-acting AMDs in specific compartments of the exposed animal, and their influence on AMR selection in different bacterial species and niches.

Finally, the choice of standardized weight in the calculation of estimated biomass denominators may be very influential on calculated AMU indicators, as demonstrated in Example 4. The FDA and ESVAC currently have differing policies related to standard cattle weights for AMU metrics, with ESVAC specifying that estimated weight at exposure should be used while FDA proposes that average weight at slaughter be used for calculations (27, 34). Since weight at slaughter will almost certainly be significantly higher than weight at exposure for cattle, if the mg/kg biomass for a cattle population calculated by ESVAC conventions were compared to mg/TAB for the same cattle population calculated by FDA conventions, the ESVAC AMU indicator would be higher than the calculated FDA antimicrobial use indicator. Clearly, standardization of animal weight used for calculation of the denominator of AMU indicators is crucial if these data are to be compared internationally, particularly if metrics are to be considered in the context of international trade.

Previous studies have also demonstrated that AMU estimates derived from the same data set can vary depending upon the metric calculated. Mills et al. (21) described the application of 5 different metrics to AMU data in dairy cattle in

TABLE 8 | Effect of use of three different durations of effect on calculations of number of animal daily doses (nADD)/100 cattle-at-risk for tulathromycin in Feedlot C.

Duration of effect	ADDkg (mg/kg/day)	Mean weight (kg)	ADD (mg/day)	Antimicrobial used (mg)	nADD	Number of cattle-at-risk	Number of cattle-at-risk/100	nADD/100 cattle
3 days	0.83	291	242.1	206,814	854.3	178,089	1,781	0.5
8 days	0.31	291	90.8	206,814	2278.2	178,089	1,781	1.3
14 days	0.18	291	51.9	206,814	3986.8	178,089	1,781	2.2

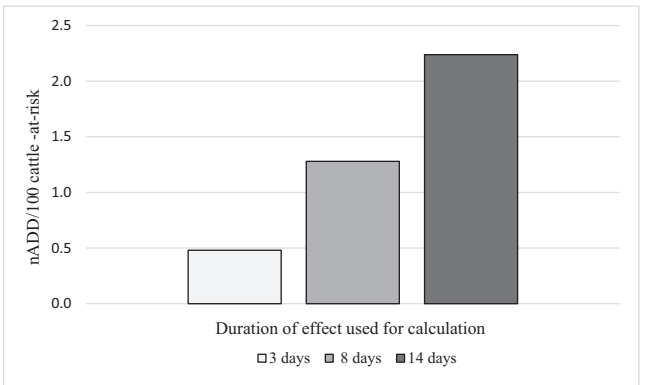

FIGURE 5 | Effect of different durations of effect (DOE) for tulathromycin on the calculation of number of animal daily doses (nADD/100 cattle-at-risk) in Feedlot C.

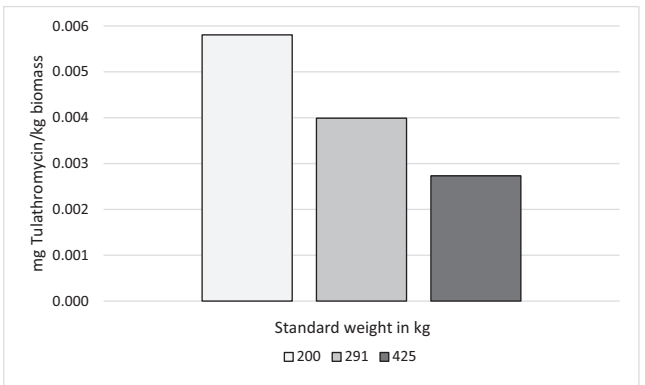

FIGURE 6 | Effect of standard weight on antimicrobial use indicators (mg tulathromycin/kg biomass)* in Feedlot C. *kg biomass = standard weight * number of cattle-at-risk.

the United Kingdom (UK): total mg, total mg/kg, daily dose, course dose, and cow calculated course. Similar to Examples 1 and 2 in the present study, these authors concluded that UK-specific AMD dosages and weights should be used for calculation of dose-based metrics and that the mg/kg indicator was only suitable for tracking AMU on a single farm when AMU patterns did not change. Similar to Example 3 in the present study, Taverne et al. (45) found that the use of different country-specific DOE correction factors for long-acting AMD in swine resulted in disparate calculations of dose-based metrics, and recommended harmonization of units of measurement to enable accurate comparisons. These studies presented examples of metric calculations in parenteral, intramammary, and in-feed data, underscoring the applicability of these concepts to AMD given by any route.

The quantification of AMU is increasingly important in both people and animals, and special features of beef cattle introduce additional challenges to an already complex venture. Regardless of the species of interest, consistency of approach (while still tailoring standards as much as possible to the study population) is of paramount importance. Clear definitions, transparent technique communication, and methodology validation are all key to the ability to compare AMU indicators between different populations of animals within species, between species, and internationally.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The protocol for this project was reviewed and approved by the Feedlot Health Management Services Ltd. Animal Care Committee (a certified holder of a Certificate of Good Animal Practice) and in accordance with standards set by the Canadian Council of Animal Care.

AUTHOR CONTRIBUTIONS

SB drafted the manuscript. SB, SH, SG, SO, CB, and PM were involved in evaluation and interpretation of AMU data, determination of relevant examples, and manuscript revision.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2019.00330/full#supplementary-material

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Antimicrobial Use on 36 Beef Feedlots in Western Canada: 2008–2012

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The accurate quantification of antimicrobial use (AMU) in production animals is critical for monitoring trends in exposure to antimicrobial drugs (AMD) over time and examining potential associations with antimicrobial resistance in bacteria. In this study, a census sample of cattle was used to quantify individually-dosed and in-feed AMU as both numbers of animal daily doses (nADD) and total grams of AMD (gAMD) used in cattle placed in 36 western Canadian feedlots between 1-November, 2008 and 31-October, 2012; representing about 21.5% of fed cattle in Canada during that time period. Of the ∼2.6 million cattle placed during the 48-month period, 45% were calves, 63% were male, 62% arrived in the fall or winter, and 39% were assessed as high risk for developing bovine respiratory disease (BRD). The proportion of cattle categorized as high risk (HR) for developing BRD was consistent over the 4 years of placement cohorts. Both medically important AMU and ionophore use were summarized but presented separately. A decrease in AMU was observed over the study period, both as nADD and total gAMD, which was primarily driven by a decline in the in-feed administration of tetracyclines. Most in-feed AMU was directed toward prevention and control of liver abscesses. The majority of individually dosed AMU was administered as metaphylaxis to address BRD risks, with category III AMD (medium importance to human medicine as categorized by Health Canada Veterinary Drugs Directorate) used most frequently. Not surprisingly, risk level for developing BRD influenced parenteral AMD exposures, with 95% of cattle categorized as being HR for developing BRD receiving individually dosed AMD compared to 59% of cattle categorized as being low risk (LR) for developing BRD. Cattle categorized as HR for developing BRD were more likely to receive macrolides for BRD metaphylaxis compared to cattle categorized as LR for developing BRD, and cattle categorized as LR for developing BRD were more likely to receive tetracycline for the same purpose. In summary, these data provide an unprecedented representation of AMU in fed cattle in western Canada and direction for future monitoring of AMU in fed cattle.

Keywords: antimicrobial use, feedlot cattle, bovine respiratory disease, Canada, quantification

INTRODUCTION

Antimicrobial drugs (AMD) are important tools for maintaining human and animal health. In North America, AMD are widely used to support feedlot cattle health. Of feedlots with more than 1,000 head of cattle in the United States, 87.5% administered AMD to animals by injection or in feed or water (1). There are concerns that widespread antimicrobial use (AMU) is an important driver of selection for antimicrobial resistance (AMR), which may be threatening the ongoing effectiveness of AMD to combat disease in people and animals (2). While AMU in any context has the potential to select for AMR, use in agricultural animals has been under increasing scrutiny due to the potential risk of transmitting resistant bacteria from animals to people by direct contact, environmental contamination, and/or consumption of contaminated meat (3–5). Because of these concerns, the concept of antimicrobial stewardship has risen to the forefront of discourse in veterinary medicine. Antimicrobial stewardship in veterinary medicine, as defined by the American Veterinary Medical Association (AVMA), "includes providing systems of care to reduce the incidence of common diseases, making evidence-based decisions about the use of AMD, and using AMD judiciously, with ongoing evaluation of the outcomes of use and consideration for animal caretakers' available resources (6)." Recognizing the particular need to preserve the effectiveness of those AMD relevant to human medicine, the World Health Organization (WHO) has published guidelines presenting evidence-based recommendations and best practice statements on the use in food animals of "medically important antimicrobials," defined as antimicrobial classes used in human medicine. Medically important antimicrobials are further categorized by the WHO according to specified criteria as "important," "highly important," or "critically important" for human medicine. The WHO recommends that the overall use of medically important AMD in food animals be reduced, with complete restriction of their use for growth promotion and in animals in which clinical disease has not been diagnosed. The WHO further suggests that critically important AMD should only be used for treatment of individual sick animals, and that highest priority critically important AMD should not be used in food animals (7). Similarly, Health Canada classifies AMD in categories I to IV based, on importance to human health (8); category I are very high importance, category II are high importance, category III are medium importance, and category IV have low importance related to public health.

Bovine respiratory disease (BRD) is the most common cause of morbidity and mortality in beef cattle, and a frequent reason for antimicrobial use (9). The microbes implicated in BRD are largely ubiquitous in cattle populations, and most of the bacterial organisms, e.g., *Mannheimia haemolytica*, *Pasteurella multocida,* and *Histophilus somni*, are normal inhabitants of the bovine upper respiratory tract (10). The likelihood of manifestation of disease in cattle is influenced by numerous factors, including host and environmental determinants, pathogen characteristics, and management practices. Cattle recently arrived at the feedlot are particularly susceptible to developing BRD. They are stressed by the transportation event, unaccustomed to their new environment, and often have been recently weaned, all of which compromise immunity. In addition, these cattle have often not been previously vaccinated against BRD pathogens, and there is frequently extensive commingling of cattle from different sources leading to exposure to infectious diseases (11).

Diagnosis in individual cattle and therefore targeted individual treatment is hampered by the tendency of cattle as prey animals to mask signs of disease and the lack of rapid, sensitive, and specific disease identification methods (12). Therefore, risk assessment for BRD is a critical component of commercial feedlot production. Risk assessment for BRD is typically done on each group of feeder cattle purchased, and the result of the BRD risk assessment is the BRD risk assignment. This assignment is based on algorithms that include factors such as age class (calf vs. yearling), body weight (often a proxy for age), procurement method (sale barn vs. ranch direct), amount of commingling before and after arrival, and previous vaccination and management history. For each assigned BRD risk category, feedlot veterinarians develop the most appropriate program for mitigating the risk of BRD. Each program includes a variety of components, including vaccination and revaccination, on-arrival antimicrobial use, biosecurity procedures, disease detection and treatment, animal husbandry practices, feeds and feeding programs, and monitoring and animal health intervention programs (9).

As defined by the AVMA, therapeutic use of antimicrobials includes applications for prevention, control and treatment of disease (6). In the context of BRD, AMD are commonly used in feedlot cattle for BRD control (i.e., metaphylaxis) in certain groups of cattle (based on their BRD risk classification) in which there are already individuals with evidence of infectious disease and for individual treatment of clinically affected animals (6, 11). The antimicrobials used in risk group protocols for on-arrival use are specifically chosen based on the level of expected exposure to infectious agents, the types of pathogens most commonly encountered (past or present), the predicted ability of the host to mount an appropriate immune response, and in some cases past research documenting efficacy of the AMD in the different cattle populations. Appropriately applied BRD metaphylaxis has been shown to dramatically reduce the deleterious effects of BRD, improving animal health (13–15) and preventing significant economic loss to producers (16).

Liver abscesses are another important health and production problem in beef cattle, with a prevalence of between 10 and 20% in most feedlots (17). Liver abscesses vary in grading from mild to severe; all liver abscesses affect animal performance to some degree with the most severe abscesses reducing the value of beef carcasses by $38 per animal (18, 19). Acidotic conditions in the rumen lead to rumenitis, allowing the establishment of bacterial infections in the ruminal wall and the subsequent translocation of pyogenic bacteria, especially *Fusobacterium necrophorum* and *Trueperella pyogenes,* via the portal circulation to the liver. Ruminal acidosis is typically associated with sudden dietary changes to high energy diets, changes in feeding patterns, overly long intervals between feedings, and feeding of too little roughage. Inclusion of tylosin in feed, the most effective of the

approved antimicrobials for liver abscess reduction incidence, has been shown by several studies to reduce liver abscesses in cattle in conventional feeding systems by 40–70% (20).

Data about AMU are collected for a variety of reasons, including the monitoring of usage trends over time, comparison of usage between different species or countries, benchmarking between hospitals, clinics, or farms, and studying the association between AMU and AMR (21). Five categories of requirements regarding measurement of AMU have been identified: level of resolution, comprehensiveness, stability of the measure over time, ability to assess exposure to AMD, and comparability of the measure between different populations. Various indicators of AMU are available and published; there can be wide discrepancies between the results obtained from different indicators applied to identical data, and no indicator fully meets all of the requirements for measurement of AMU. Selection of the appropriate indicator requires consideration of the study objectives and determination of which indicator best meets the needs of the study (21). To fully understand the role AMU plays in the selection of AMR in feedlot cattle, and to measure the potential effect of interventions, accurate AMU data must be available (22). Often, AMD sales data have been used as a proxy for AMD administration (23, 24). It is important, however, to recognize that sales data are not equivalent to use data and may result in use overestimation, because producers may not administer what is purchased. Furthermore, it is not always possible to correctly attribute the species in which a product is used because AMD are often authorized for use in multiple species. Detailed farm-level AMU data collection is therefore considered invaluable due to its more closely targeted nature, ensuring accurate assignation of species exposed to the AMD, ability to evaluate the indication of use and risk characteristics of exposed animals, and potential exploration of relationships of AMU with AMR in a meaningful way (25). Since AMU data collected for monitoring and surveillance are intended to address questions requiring detailed levels of information (26, 27) development of practical methods for on-farm collection of data should be prioritized.

In this study, a census sample of >2.6 million cattle entering feedlots in western Canada over ∼4 years was performed to summarize AMU in this sector, increase knowledge of the indications for which AMD are administered, and evaluate trends in AMU over time. In addition to providing an unprecedented representation of AMU in fed cattle in western Canada, this study also sought to evaluate the development of methods for feedlot AMU monitoring.

MATERIALS AND METHODS

Project Summary

Detailed data about AMD administered to cattle in 36 western Canadian feedlots from November 1, 2008 to October 31, 2012, including information about the specific AMD, dose administered, use indication, and exposed animal characteristics/demographics were collected. The AMU data were converted to indicators of frequency or numbers of animal daily doses (nADD) (28) or grams AMD (gAMD) per 100,000 cattle. Data were summarized and statistical analyses performed to determine relative risks of exposure to AMD or confidence intervals for binomial proportions where appropriate.

Feedlots and Animals

Mixed-breed cattle placed in 36 western Canadian feedlots and fed for beef production from November 1, 2008 to October 31, 2012 (n = 2,615,082) were enrolled in the study. The cattle were divided into 4 placement cohorts (PC1, PC2, PC3, and PC4) based on date of arrival into the feedlot. Placement cohort 1 (PC1) included cattle arriving between November 1, 2008 and October 31, 2009, PC2 included cattle arriving between November 1, 2009 and October 31, 2010, PC3 included cattle arriving between November 1, 2010 and October 31, 2011, and PC4 included cattle arriving between November 1, 2011 and October 31, 2012. Cattle were owned and managed by multiple individuals and companies, but their healthcare was overseen by a single veterinary practice (Feedlot Health Management Services Ltd; Feedlot Health), who worked with feedlots to develop risk assessment algorithms and risk-based animal health and treatment protocols. This study population represented ∼21.5% of fed cattle in Canada for the time period. The one-time capacity of 8 of the feedlots was <5,000 cattle, 5,000–9,999 for 15 of the feedlots, 10,000–20,000 for 5 of the feedlots, and >20,000 cattle for 8 of the feedlots.

The basic design of the feedlots and management strategies were representative of typical operations in western Canada; animals were housed in open air, dirt-floor pens arranged side by side with central feed alleys. Designated animal handling facilities with a hydraulic chute, individual animal scale, and chute-side computer for data recording were located at each site. Standardized animal health and treatment protocols developed by Feedlot Health directed parenteral and oral bolus AMD exposures of cattle, and prescriptions for in-feed AMD were written for feedlots by Feedlot Health veterinarians with valid veterinarian-client-patient relationships.

Data Collection and Management

Using proprietary data collection and management software (*i*FHM*S*, Feedlot Health, Okotoks, AB), individual animal data were collected at initial processing and subsequent handling times. Individual animal identification included both a Canadian Cattle Identification Agency approved electronic tag (national ID) and a color-coded, uniquely numbered dangle ear tag (visual ID), with both tags recorded and correlated to the individual animal in the database. Data were compiled for analysis using Microsoft® Access 2010 (Microsoft Corporation, Redmond, WA) and Microsoft® Excel 2010. Information collected at arrival included date, unique animal identification number, sex, age category (calf or yearling), feedlot number, and production lot number. A production lot was defined as a group of cattle purchased together with similar characteristics. Risk assessment for BRD and assignment of status (high or low risk for development of BRD) was automatically performed by *i*FHM*S* at time of placement based on historical data and customized, if necessary, by veterinarians and other personnel working under their supervision. Information

collected included unique identification number, date, animal weight at time of administration, active AMD ingredient, dosage, route, reason for administration (metaphylactic or treatment), and disease/syndrome [acute interstitial pneumonia/diphtheria, undifferentiated fever/BRD, lameness (arthritis, footrot, foot lesions), metabolic disease (bloat, grain overload, laminitis), nervous disease, eye disease, and other].

Data regarding in-feed AMU were assembled using a combination of approaches including Feedlot Health veterinarian feed AMD prescriptions, daily feed delivery data previously collected by Feedlot Health and stored in a database for consulting purposes, extraction of feed data from the feedlot's on-site computer system, obtaining a hard copy of the feed delivery records, or through combinations of these approaches. Data including unique production lot/feedlot combinations, feed delivery date, number of animals in the production lot each day, and number of animals receiving each type of in-feed AMD were compiled into Microsoft® Excel 2010. In-feed AMU was either reported as number of animals receiving a certain inclusion rate of AMD in feed (e.g., 35 mg chlortetracycline per kg dry matter) or the number of animals receiving a certain number of grams per head (e.g., 1 gram of chlortetracycline per head per day). Although cattle within a production lot could arrive over a span of days, they were typically assigned to the same placement cohort if the group arrived between 1 November and 31 October of the following year. However, if the production lot happened to arrive at the juncture of placement cohorts (i.e., was placed between 30 October and 2 November of the same year), the production lot was divided between the cohorts. In these cases, feed data were prorated to the appropriate placement cohort based on the percentage of animals in the production lot assigned to each placement cohort. Feed data were prorated according to risk category for BRD (high or low) according to the assessed BRD risk status of the animal at entry into the feedlot.

Data were summarized and metrics/indicators calculated using SAS® software (Windows version 9.4, SAS Institute, Cary, NC). For this study, AMD classified in categories I to III, or medium to very high importance to human medicine (8), are summarized and presented separately from category IV AMD of low importance to human medicine (e.g., ionophores).

Metrics and Indicators

Metrics summarized included total gAMD and number of nADD for both individually dosed AMD (parenterally or orally administered) and AMD administered in feed to entire housing groups. Frequency of exposure (e.g., number of cattle exposed) was also summarized where appropriate.

For individually dosed AMD, total gAMD used were calculated by summing the administered mg of AMD recorded per animal. For AMD administered in feed at a given inclusion concentration, the mg of AMD per kg of feed was multiplied by the estimated average intake of daily feed per animal (29) to calculate the estimated daily intake of AMD per animal. This intake was then multiplied by the number of animals fed the ration daily to yield the amount of the particular AMD used. For AMD fed on a mg/animal basis, this dose was multiplied by the number of animals exposed to yield the daily amount of AMD used.

To calculate the number of standardized doses of individually administered AMD, an ADD_{kg} was assigned for each drug in mg/kg/day (Individually Dosed AMD Appendix, **Supplementary Material**). Use of tetracyclines, macrolides, fluoroquinolones, phenicols, cephalosporins, penicillin, sulfonamides, and potentiated sulfonamides was recorded. For long-acting AMD (effect lasting longer than 24 h), the administered mg/kg dosage was divided by the number of days of duration that a single dose of a particular product is assumed to maintain therapeutic concentrations in the target tissues to produce the ADD_{kg}, based on pharmacokinetics and pharmacodynamics studies and the product label. An individual animal weight was recorded for ∼95% of parenteral or oral bolus treatment, and a group average weight was recorded for 4.7% of these exposures. No weight was recorded for the remainder of exposures; in these instances, the mean weight at exposure was calculated for the type of AMD and this weight substituted. The calculation of the number of animal daily doses (nADD) of individually dosed AMD was performed for each administration via SAS software using the following equation:

$$nADD = \frac{Qty\ of\ active\ substance\ in\ mg\ administered}{ADD\ (mg\ per\ kg\ per\ day)^{*}weight\ \left(kg\right)\ of\ animal}$$

Calculations of the standardized doses for in-feed AMD were performed differently since the AMD were mixed in feed to target either mg of drug/kg dry matter of feed consumed or mg of drug administered per animal per day, and the number of cattle consuming the ration daily was recorded. Use of tetracyclines, tylosin, and ionophores was recorded in feed. For AMD where there was not a range of dosages administered across feedlots, 1 ADD was equal to 1 animal recorded as exposed to the in-feed AMD. In instances with a range of dosages, relative nADD were calculated by normalizing to the highest dose used. In instances with an inclusion rate based on mg per kg dry matter consumed, nADD were standardized based on feed intake estimates (In Feed AMD Appendix, **Supplementary Material**). For in-feed administration of chlortetracycline, which had the widest range of dosages reported for various indications, the average of the reported dosage range for metaphylaxis or treatment of *Histophilus somni* was used as the reference ADD, instead of the highest dose. For in-feed tylosin exposures, the in-feed dosage was normalized by the parenteral dosage labeled for use in respiratory disease in cattle.

Treatment frequency with an AMD was calculated as nADD/100,000 cattle. When the total number of animals in the population is the denominator, treatment frequency indicates how many days on average an animal in the population is treated with an AMD during the time of data collection (30). With these data, if the number of animals in the population was used as the denominator, most of the indicators would have been <0 and tables would have been difficult to read. Therefore, for convenience and easier reading, the denominator was multiplied by 100,000. Thus, treatment frequency in this

TABLE 1 | Characteristics of cattle by placement cohort[a], cattle placed 2008–2012.

	Placement cohort				
	1	2	3	4	Total
	n = 717,176	*n* = 670,066	*n* = 648,916	*n* = 578,924	*n* = 2,615,082
Characteristics					
AGE AT ARRIVAL, NO. (%)					
Calf	333,742 (47)	314,190 (47)	288,484 (44)	244,083 (42)	1,180,499 (45)
Yearling	383,434 (53)	355,876 (53)	360,432 (56)	334,841 (58)	1,434,583 (55)
SEX, NO. (%)					
Male	453,222 (63)	399,396 (60)	420,739 (65)	370,171 (64)	1,643,528 (63)
Female	263,954 (37)	270,670 (40)	228,177 (35)	208,753 (36)	971,554 (37)
SEASON OF ARRIVAL, NO. (%)					
Fall or Winter	424,138 (59)	413,518 (62)	412,678 (64)	366,352 (63)	1,616,686 (62)
Spring or Summer	293,038 (41)	256,548 (38)	236,238 (36)	212,572 (37)	998,396 (38)
BRD[b] RISK CATEGORY, NO. (%)					
High	269,404 (38)	265,033 (40)	259,667 (40)	227,535 (39)	1,021,639 (39)
Low	447,772 (62)	405,033 (60)	389,249 (60)	351,389 (61)	1,593,443 (61)

The number of cattle are presented in the left part of the cell with the % of the total number of cattle in the placement cohort this number represents in the right part of the cell, in parentheses. Percentages of use may not add to 100% due to rounding.

[a]*Placement cohort comprised of cattle placed in the feedlot between 1 November and 31 October of consecutive years.*

[b]*Bovine Respiratory Disease.*

context represents how many days on average 100,000 animals were treated with an AMD during the feeding period. Where suitable, the gAMD/100,000 cattle indicator was also calculated for comparison.

Statistics

Relative risks of exposure to AMD were estimated using Poisson regression (Proc GENMOD, SAS v9.4, SAS Inc., Cary, NC) as previously described (31) using numbers of AMD exposures as the dependent variable and the assessed risk category of the animal for BRD as the independent variable. Robust error variances were estimated using the repeated statement and the individual identification number of the animal as the subject identifier. For estimation of percentages of cattle exposed to AMD for different reasons (**Table 7**), width-adjusted 95% confidence intervals (95% CI) for binomial proportions were calculated, adding 2 successes and 2 failures to actual counts as previously described (32).

RESULTS

Demographics of the Cattle

Approximately 2.6 million cattle entered the 36 feedlot study sites over the 4-year period of the study and were followed until feedlot exit (**Table 1**). While the number of animals in each placement cohort slightly decreased over the study period, the placement numbers were fairly consistent from year to year. Overall, more males (63%) than females and more yearlings (55%) than calves were included in this study population, and the majority of animals entered the feedlot in the fall or winter (62%) and were classified as low risk (LR) for developing BRD (61%). The BRD risk category of the cattle placed over time was consistent [∼39% categorized as high risk for developing BRD (HR)]. The cattle in these 36 feedlots in these 4 placement cohorts comprised 21.5% of the cattle fed during the same time period in Canada. Cattle assessed to be at HR for developing BRD tended to be calves that entered the feedlot in the fall or winter (**Table 2**). Sex of cattle did not appear to significantly influence risk categorization for BRD.

TABLE 2 | Characteristics of cattle overall and stratified by risk for bovine respiratory disease (BRD) assessed at placement, cattle placed 2008–2012.

	Overall	High risk for BRD	Low risk for BRD
	n = 2,615,082	*n* = 1,021,639	*n* = 1,593,443
Characteristics			
AGE AT ARRIVAL, NO. (% OF TOTAL, % OF BRD RISK GROUP)			
Calf	1,180,499 (45)	950,197 (36, 93)	230,302 (9, 14)
Yearling	1,434,583 (55)	71,442 (3, 7)	1,363,141 (52, 86)
SEX, NO. (% OF TOTAL, % OF BRD RISK GROUP)			
Male	1,643,528 (63)	662,896 (25, 65)	980,632 (37, 62)
Female	971,554 (37)	358,743 (14, 35)	612,811 (23, 38)
SEASON OF ARRIVAL, NO. (% OF TOTAL, % OF BRD RISK GROUP)			
Fall or Winter	1,616,686 (62)	836,927 (32, 82)	779,759 (30, 49)
Spring or Summer	998,396 (38)	184,712 (7, 18)	813,684 (31, 51)

The number of cattle with the characteristic is presented in the left part of the cell. For the Overall column, the % of cattle that this number represents is presented in the right part of the cell in parentheses. For the High Risk for BRD and Low Risk for BRD columns, in parentheses and separated by a comma, first the % of total cattle is presented, then the % of cattle in that risk category is presented. Percentages of use may not add to 100% due to rounding.

TABLE 3 | Individually dosed and in-feed antimicrobial drug use (AMU) in number of animal daily doses (nADD) and total grams of antimicrobial drug (gAMD) by placement cohort[a], cattle placed 2008–2012.

		Placement cohort				
		1 (*n* = 717,176)	2 (*n* = 670,066)	3 (*n* = 648,916)	4 (*n* = 578,924)	Total (*n* = 2,615,082)
nADD or gAMD, NO. (NO./100,000 CATTLE)						
Individual	nADD	1,680,387 (234,306)	1,532,732 (228,743)	1,383,193 (213,154)	1,226,748 (211,901)	5,823,060 (222,672)
	gAMD	3,674,494 (512,356)	3,462,134 (516,685)	2,899,241 (446,782)	2,611,049 (451,018)	12,646,918 (483,615)
In Feed	nADD	8,300,631 (1,157,405)	7,539,570 (1,125,198)	7,105,901 (1,095,042)	5,744,496 (992,271)	28,690,598 (1,097,120)
	gAMD	46,488,463 (6,482,155)	42,304,541 (6,313,489)	39,735,901 (6,123,428)	32,315,393 (5,581,975)	160,844,298 (6,150,641)

The total nADD or gAMD is presented on the left side of the cell, and the nADD/100,000 cattle or gAMD/100,000 cattle is presented on the right side of the cell in parentheses.
[a]*Placement cohort comprised of cattle placed in the feedlot between 1 November and 31 October of consecutive years.*

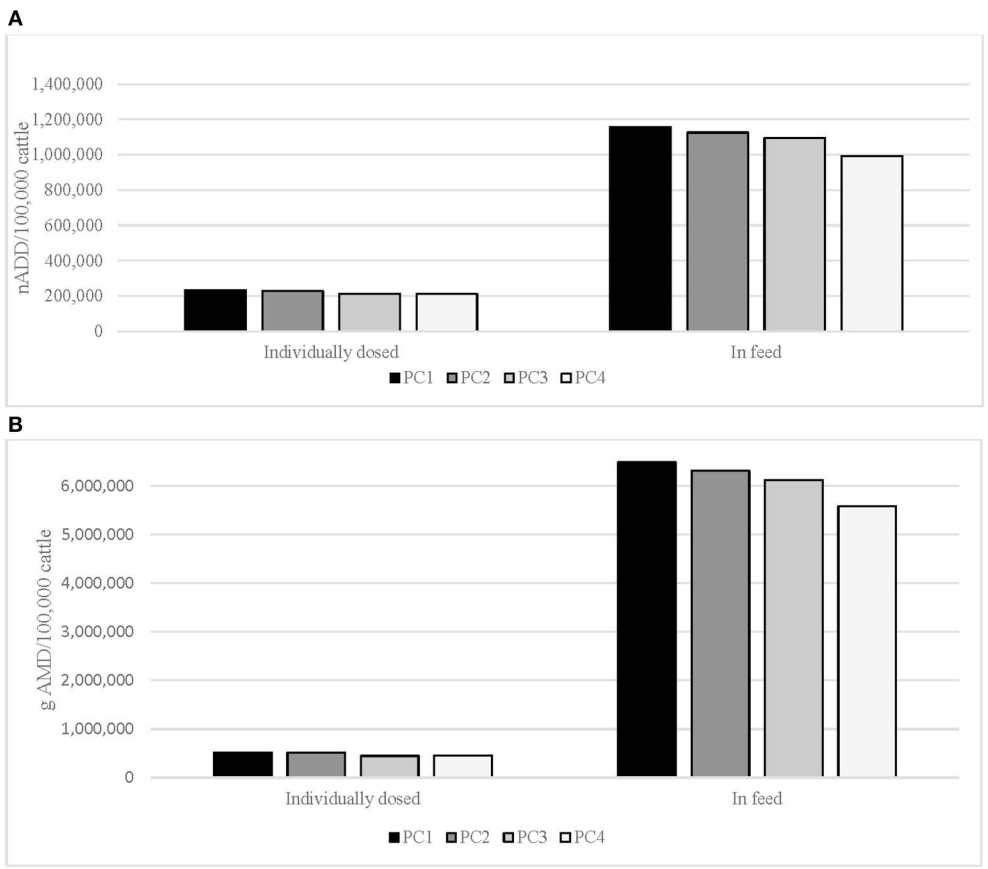

FIGURE 1 | (A) Individually dosed and in-feed antimicrobial drug use (AMU) in number of animal daily doses (nADD)/100,000 cattle in placement cohort[a] (PC), cattle placed 2008–2012. **(B)** Individually dosed and in-feed antimicrobial drug use in total grams of antimicrobial drug (gAMD)/100,000 cattle in placement cohort[a] (PC), cattle placed 2008–2012. [a]Placement cohort comprised of cattle placed in the feedlot between 1 November and 31 October of consecutive years.

Overall AMU

Substantially more medically important AMD were used in-feed than dosed individually, whether measured with the nADD/100,000 cattle or total gAMD/100,000 cattle metric (**Table 3**, **Figures 1A,B**). When calculated with nADD, 5 times as much medically important AMD were used in-feed than was administered to individual cattle through the study period; when gAMD were used as the metric, almost 13 times as much AMD was used in-feed. A reduction in individually dosed (average 11%) and in-feed medically important AMU (average

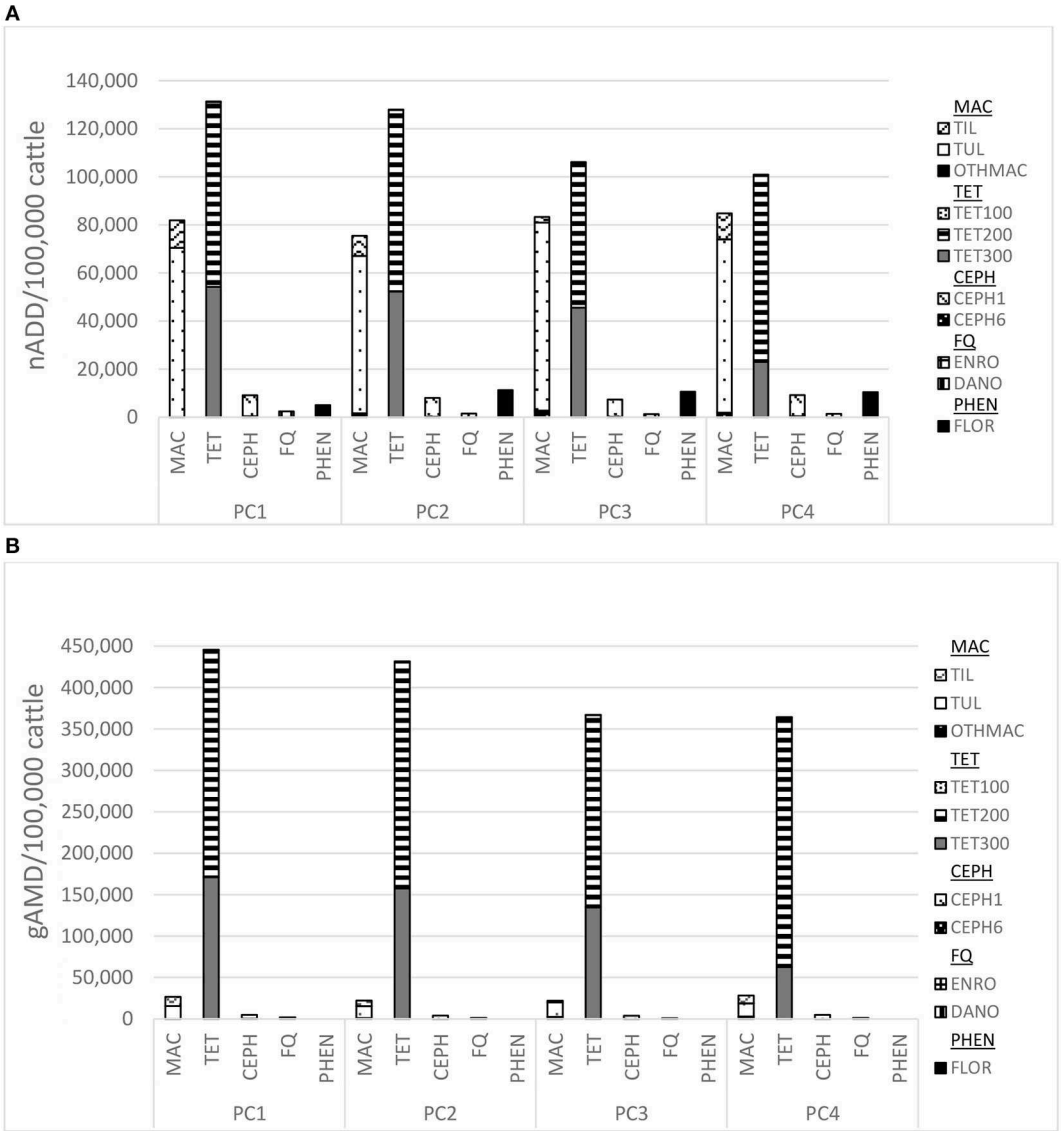

FIGURE 2 | (A) Individually dosed antimicrobial drug use in nADD/100,000 cattle by placement cohort (PC)[a], antimicrobial class[b], and specific type of antimicrobial drug[c], cattle placed 2008–2012. **(B)** Individually dosed antimicrobial drug (AMD) use in grams AMD (gAMD)/100,000 cattle by placement cohort (PC)[a], antimicrobial class[b], and specific type of antimicrobial drug[c], cattle placed 2008–2012. [a]Placement cohort comprised of cattle placed in the feedlot between 1 November and 31 October of consecutive years. [b]MAC, macrolides; TET, tetracyclines; CEPH, third generation cephalosporins; FQ, fluoroquinolones; PHEN, phenicols (TMS, trimethoprim-sulfamethoxazole; PEN, penicillin; SULF, sulfonamides not depicted due to low usage; [c]TIL, tilmicosin 10 mg/kg; TUL, tulathromycin 2.5 mg/kg; OTHMAC, gamithromycin 6 mg/kg, tildipirosin 4 mg/kg, tylosin 29 mg/head; TET100, oxytetracycline 6.67 mg/kg; TET200, oxytetracycline 20 mg/kg; TET300, oxytetracycline 30 mg/kg; CEF1, ceftiofur hydrochloride or sodium, 1.1 mg/kg; CEF6, ceftiofur crystalline free acid 6.6 mg/kg; DANO, danofloxacin 6 mg/kg; ENRO, enrofloxacin 7.7 mg/kg; FLOR, florfenicol 40 mg/kg.

14%) over the study period was evident using both indicators (**Figures 1A,B**).

AMU by Drug Class and Type of AMD

Employing the nADD/100,000 cattle indicator (**Figure 2A**), tetracyclines were the class of AMD most commonly administered to individual cattle (52.8% of total individually dosed usage). Macrolides were the second most common AMD class administered to individual cattle (36.5%), with tulathromycin constituting the majority of this antimicrobial class use (88.0%). While tetracycline use decreased over time by 23.1%, macrolide use slightly increased over the course of the study (3.6%). Macrolide use appeared markedly lower when assessed using the gAMD/100,000 cattle metric (**Figure 2B**); based on this indicator tetracycline use comprised 83.5% of individually dosed AMU while macrolides represented only 5%.

Employing either the nADD/100,000 cattle or gAMD/100,000 cattle metric, the majority of medically important AMD

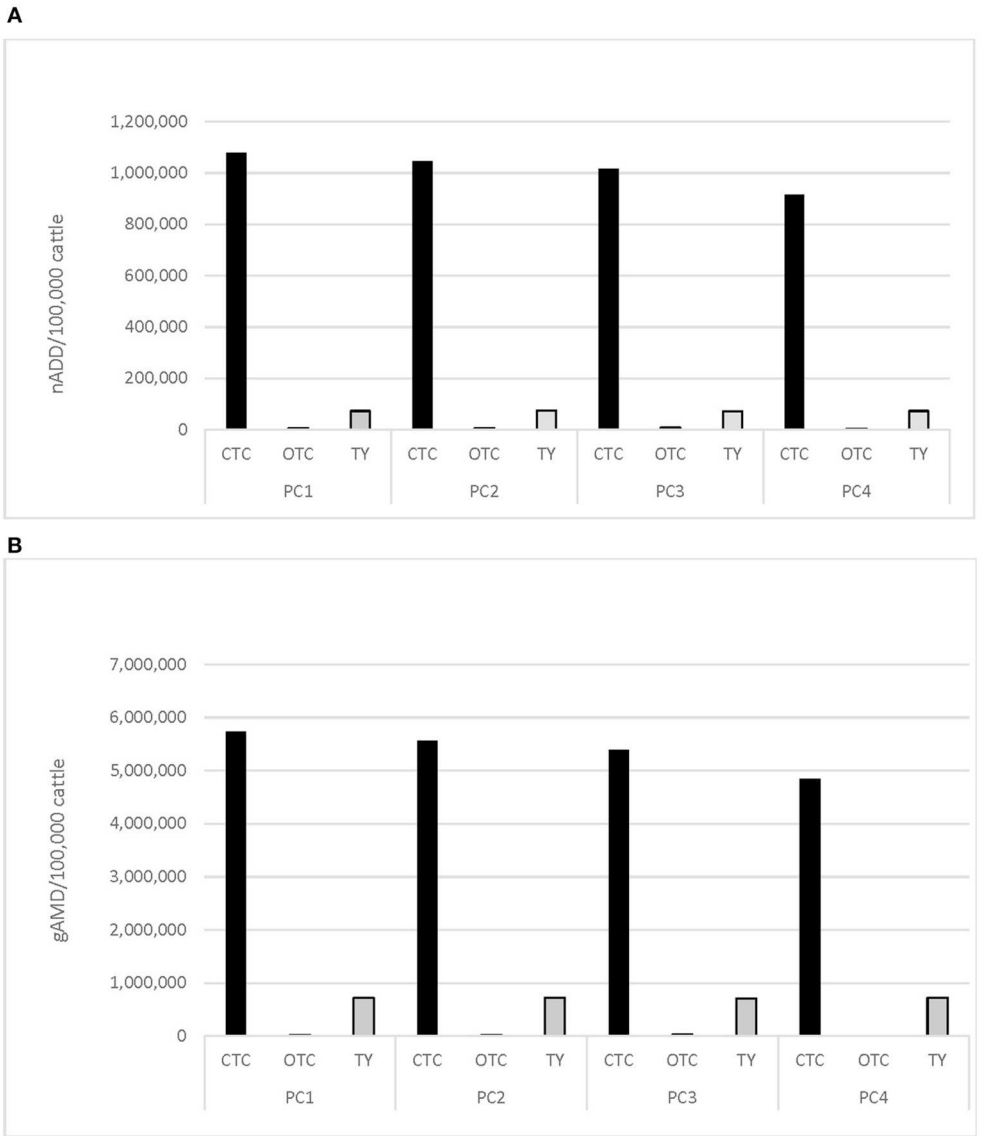

FIGURE 3 | (A) In-feed antimicrobial drug use in nADD/100,000 cattle by placement cohort (PC)[a], and antimicrobial class[b], cattle placed 2008–2012. **(B)** In-feed antimicrobial drug use in grams AMD (gAMD)/100,000 cattle by placement cohort (PC)[a], and antimicrobial class[b], cattle placed 2008–2012.
[a]Placement cohort comprised of cattle placed in the feedlot between 1 November and 31 October of consecutive years. [b]CTC, chlortetracycline; OTC, oxytetracycline; TY, tylosin.

administered in-feed was tetracycline (**Figures 3A,B**), followed by tylosin. When the nADD indicator was employed, there was 14 times as much tetracycline (chlortetracycline and oxytetracycline) used as tylosin; when the gAMD indicator was employed, there was 7.5 times as much tetracycline used as tylosin over the course of the study.

AMU by Indication, Risk Class for BRD, and Cohort

Overall, using nADDs, category III AMD comprised the greatest amount of individually dosed AMU (56.9%); 38.5% of individually dosed AMU were category II AMD and 4.6% of individually dosed AMU were category I AMD (**Table 4**). The bulk of individually dosed AMU (92.9%) was administered to prevent or treat BRD occurrence, with the majority of this use (89.9%) administered as BRD metaphylaxis. Of the category I AMD use, 83.2% was comprised of third-generation cephalosporins; 44.3% was administered to treat infectious causes of lameness and 24.3% was used to treat other miscellaneous infectious diseases (ocular diseases, infectious neurologic disorders, etc.).

Most individually dosed AMD administered for BRD metaphylaxis were tetracycline class drugs (59.3%), followed by macrolides (40.7%) and a small amount of phenicol class drugs (**Table 5**). Cattle assessed to be at HR for developing BRD were about 1.6 times more likely to receive metaphylaxis for BRD than LR cattle, and about 4 times as likely to be treated

TABLE 4 | Individually dosed antimicrobial use by indication[a,b] and class of antimicrobial drug (AMD)[c], organized by category of importance to human medicine[d] and expressed in number of animal daily doses (nADD).

	Indication						
	ARD	UF/BRD		Lameness	Other	Implant	Total
AMD Class		Metaphylaxis	Treatment				
	nADD No. (% of use of specific AMD class, % of use for specified indication)						
I FQ	3 (0.01, 0.01)	0 (0, 0)	44,192 (99.4, 7.9)	71 (0.2, 0.03)	181 (0.4, 0.1)	0 (0, 0)	44,447
CEPH	33,075 (15.0, 98.7)	0 (0, 0)	51,498 (23.3, 9.2)	99,515 (45.1, 44.3)	36,604 (16.6, 24.3)	0 (0, 0)	220,692
II MAC	85 (0.004, 0.25)	1,975,173 (93.0, 40.7)	143,019 (6.7, 25.6)	602 (0.03, 0.3)	2,579 (0.1, 1.7)	3,061 (0.1, 100)	2,124,519
PEN	3 (0.2, 0.01)	0 (0, 0)	13 (0.8, 0.002)	1,347 (81.7, 0.6)	286 (17.3, 0.2)	0 (0, 0)	1,649
TMS	214 (0.2, 0.6)	0 (0, 0)	659 (0.6, 0.1)	72,733 (62.8, 32.4)	42,146 (36.4, 28.0)	0 (0, 0)	115,752
III TET	64 (0.002, 0.2)	2,876,561 (93.6, 59.3)	81,634 (2.7, 14.6)	49,860 (1.6, 22.2)	64,933 (2.1, 43.2)	0 (0, 0)	3,073,052
PHEN	74 (0.03, 0.2)	584 (0.2, 0.01)	238,256 (98.6, 42.6)	360 (0.1, 0.2)	2,258 (0.9, 1.5)	0 (0, 0)	241,532
SULF	0 (0, 0)	0 (0, 0)	20 (1.4, 0.003)	8 (0.6, 0.004)	1,389 (98.0, 0.9)	0 (0, 0)	1,417
ALL	33,518	4,852,318	559,291	224,496	150,376	3,061	5,823,060

The nADD is presented on the left side of the cell. In parentheses on the right side of the cell, the % of use of the specific AMD class is first presented, then the % of use for specified indication presented separated by a comma. Percentages of use may not add to 100% due to rounding. Darkening green color indicates increasing nADD.

[a]ARD, acute respiratory disease; UF/BRD, undifferentiated fever/bovine respiratory disease; Other, neurologic, metabolic, ocular, or other indications; Implant, antimicrobial associated with implantation of hormone.

[b]Metaphylaxis, group administration of an antimicrobial to a population at risk for disease before overt clinical disease is apparent in the entire group; Treatment, administration of an antimicrobial to an individual animal diagnosed with clinical disease.

[c]FQ, fluoroquinolones; CEPH, 3rd generation cephalosporins; MAC, macrolides; PEN, penicillins; TMS, trimethoprim-sulfamethoxazole; TET, tetracyclines; PHEN, phenicols; SULF, sulfonamides.

[d]Roman numerals I to III signify categories of importance to human medicine as designated by the Canadian Veterinary Drugs Directorate.

for BRD (**Tables 5**, **6**). High risk cattle were over 100 times more likely to receive a macrolide for BRD metaphylaxis than cattle assessed to be LR. Conversely, HR cattle were about a third less likely to receive tetracycline for BRD metaphylaxis than LR cattle. Of the AMD used for treatment of BRD, phenicols comprised the highest percentage (42.6%); HR cattle were 2.3 times more likely to receive a phenicol for this purpose than LR cattle. Other AMD used for treatment of BRD, listed in descending amount of usage, were macrolides (25.6%), tetracyclines (14.6%), cephalosporins (9.2%), fluoroquinolones (7.9%), potentiated sulfonamides, sulfonamides, and penicillin (all <0.01).

Most in-feed medically important AMU was related to prevention and treatment of liver abscesses (59.5%) and prevention and treatment of histophilosis (40.2%) (**Table 7**). Of in-feed medically important AMU, 92.8% was chlortetracycline (category III AMD), comprising 100% of use for histophilosis, 88.1% of use for liver abscesses, and about half of use for "other" indications. Macrolides (category II AMD) were only used for prevention and treatment of liver abscesses and made up 11% of AMU for this purpose.

Overall, throughout the course of the study 97% of cattle were exposed to medically important AMD in feed, 73% were individually dosed with AMD, and 21% received tylosin as part of a hormonal growth implant. The percentage of cattle exposed to AMD in-feed or as part of growth implants did not appear to be influenced significantly by age, sex, season of arrival, or assessed risk category for BRD (range of exposure 95–98% for in-feed AMD and 19–24% for implants). Conversely, higher percentages of cattle that were calves, male, arriving in cold weather, and assessed to be at HR for developing BRD were exposed to individually dosed AMD (**Table 8**); 95% of cattle assessed to be at HR for developing BRD were exposed to individually dosed AMD compared to 59% of LR cattle.

Over 1.8 million cattle were exposed individually for metaphylaxis or treatment of BRD; 70.1% of cattle overall received individually dosed metaphylaxis for BRD and 5.9% were treated individually for BRD (**Table 9**). The percentage of cattle receiving individually dosed BRD for metaphylaxis and treatment both decreased slightly over the course of the study (2.9 and 1.3%, respectively). The percentage of cattle receiving AMD treatment for reasons other than BRD increased slightly (0.3%).

Considering the use of class I AMD over time (**Table 10**), the use of fluoroquinolones decreased from 2,442 ADD/100,000 cattle at risk to 1,448 ADD/100,000 cattle at risk (40.7%) while the use of cephalosporins decreased from PC1 to PC3 from 9,135 ADD/100,000 cattle at risk to 7,379 ADD/100,000 cattle at risk (19%), but then increased in PC4 back to the PC2 level. Class I AMD were all individually administered and comprised 0.8% of all medically important AMU (in-feed and individually dosed). For class II AMD, the use of individually dosed macrolides increased very slightly from PC1 to PC4 (3.2%) but use over time appeared to remain fairly consistent as the overall average use was similar to that used by PC1. Similarly, in-feed macrolide use remained consistent over the course of the study. The use of penicillin

TABLE 5 | Individually administered antimicrobial use by antimicrobial class[a] for bovine respiratory disease (BRD) in total number of animal daily doses (nADD), stratified by reason for exposure, and risk category for BRD with relative risk of antimicrobial exposure for cattle assessed to be high risk (HR) or low risk (LR) for BRD.

		Reason for Exposure		Assessed risk category for BRD	
		Metaphylaxis[b]	Treatment[c]	HR	LR
	AMD Class	nADD (% of total for column)			
I	FQ	0	44,192	34,713	9,479
		(0)	(7.9)	(1.0)	(0.5)
	CEPH	0	51,498	22,911	28,587
		(0)	(9.2)	(0.7)	(1.4)
II	MAC	1,975,173	143,019	2,049,808	68,384
		(40.7)	(25.6)	(60.9)	(3.3)
	PEN	0	13	13	0
		(0)	(<0.01)	(<0.01)	(0)
	TMS	0	659	131	528
		(0)	(<0.01)	(<0.01)	(<0.01)
III	TET	2,876,561	81,634	1,060,891	1,897,304
		(59.3)	(14.6)	(31.5)	(92.8)
	PHEN	584	238,256	198,043	40,797
		(0)	(42.6)	(5.9)	(2.0)
	SULF	0	20	16	4
		(0)	(<0.01)	(<0.01)	(<0.01)
	ALL	4,852,318	559,291	3,366,526	2,045,083
		(100)	(100)	(100)	(100)

The nADD is presented in the top of the cell, and the % of the total nADD by reason or assessed risk category for BRD that this number represents is presented in parentheses in the bottom of the cell. Percentages of use may not add to 100% due to rounding.
[a]FQ, fluoroquinolones; CEPH, 3rd generation cephalosporins; MAC, macrolides; PEN, penicillin; TMS, trimethoprim-sulfamethoxazole; TET, tetracyclines; PHEN, phenicols; SULF, sulfonamides. Roman numerals I to III signify category of importance to human medicine as designated by the Canadian Veterinary Drugs Directorate.
[b]Metaphylaxis is the group administration of an antimicrobial to a population at risk for disease before overt clinical disease is apparent in the entire group.
[c]Treatment is the administration of an antimicrobial to an individual animal diagnosed with clinical disease.

and potentiated sulfonamides was low and stable. When use of in-feed and individually dosed medically important AMD were summed, class II AMD use comprised 12% of all AMD use. Summed in-feed and individually dosed class III AMD use made up 87% of all medically important AMD use, with the majority of this being tetracyclines (99% overall and 90% of medically important in-feed use). Over time, the use of individually dosed tetracycline and in-feed tetracycline decreased significantly overall from 1,215,633 ADD/100,000 cattle at risk to 1,020,057 ADD/100,000 cattle at risk (16.1%; 23.1% individually dosed and 15.2% in feed). Individually dosed phenicol use doubled, while sulfonamide use was both light and decreased over time.

Examining trends over time in specific in-feed AMU (**Table 11**), the use of tetracyclines for prevention and treatment of respiratory disease (histophilosis) decreased over time from 500,713 ADD/100,000 cattle at risk to 326,174 ADD/100,000 cattle at risk (34.9%). In feed AMU for liver abscess prevention was consistent over the course of the study for both tetracyclines and macrolides. The use of tetracyclines for other indications (i.e., pododermatitis and keratoconjunctivitis) comprised only a small amount of overall in-feed use (0.3%) and varied from cohort to cohort.

Ionophore Use

The ionophores monensin and lasalocid were used during the study. Using nADD, the use of ionophores comprised >89% of in-feed AMU overall (medically and non-medically important). Monensin was the most widely fed ionophore, constituting 99.9% of total use (**Table 12**), and use was consistent over time from cohort to cohort.

DISCUSSION

The comprehensiveness and scope of this study provide an unprecedented representation of AMU in the Canadian feedlot sector in a large population of cattle managed by the same veterinary practice. While these data would ideally encompass a more recent period for the most timely estimates and descriptions of use, they nevertheless provide a baseline and practical information about methodological approaches. The thorough data collection allowed for not only an examination of general AMU trends, but also detailed evaluation of reasons for use and specific characteristics of exposed cattle.

Overall, if all AMD categories were considered together, the use of category IV (non-medically important) ionophores in feed comprised the majority of AMU in this population of beef cattle on an nADD basis. This fact underscores the importance of transparency in reporting AMD categories in AMU. These data demonstrate the huge potential for variability in the summary measures for AMU in beef cattle, depending upon inclusion or exclusion of ionophores. In this dataset, if ionophore use (non-medically important AMD) had been aggregated with category I through III AMU (medically important AMD), AMU would have been nearly 10 times that which was reported without category IV AMD. This would have obvious implications to users of these data if AMU was to be compared among groups, with some groups including ionophores in aggregate summaries and others not. When only medically important AMD (both in-feed and individually dosed) were considered (**Table 10**), the preponderance of use (almost 90% of medically important AMD) was category III AMD (8). Category I AMD (fluoroquinolones and ceftiofur) represented only a small fraction (<1%) of the medically important AMD used in feedlots; in addition, all category I AMD were individually dosed, and their use decreased over time. This is an encouraging sign that current practices in the feedlot industry support good antimicrobial stewardship, in that AMD of lesser importance to human medicine are being selected when feasible and effective (7). However, macrolides (category II AMD) still comprised ∼12% of use, and their use remained fairly consistent throughout the years, suggesting that continued focus on antimicrobial stewardship in this area is essential. Of note, the WHO

TABLE 6 | Output of univariate regression analysis for estimates of relative risk of exposure to specific antimicrobial drugs (AMD) for indications of metaphylaxis[a] or treatment[b] of bovine respiratory disease (BRD) in cattle classified as high risk (HR) for developing BRD and cattle classified as low risk (LR) for developing BRD.

AMD	Indication	Regression coefficient	*p* value	Relative risk estimate	95% confidence interval
Any	BRD (Metaphylaxis)	0.49	<0.0001	1.63	1.629–1.634
	BRD (Treatment)	1.40	<0.0001	4.07	4.025–4.114
Macrolide	BRD (Metaphylaxis)	4.62	<0.0001	101.55	98.976–104.199
	BRD (Treatment)	−0.16	<0.0001	0.85	0.838–0.867
Tetracycline	BRD (Metaphylaxis)	−1.04	<0.0001	0.35	0.352–0.354
	BRD (Treatment)	0.08	<0.0001	1.08	1.057–1.010
Phenicol	BRD (Metaphylaxis)	−0.26	0.07	0.77	0.583–1.023
	BRD (Treatment)	0.84	<0.0001	2.31	2.273–2.348

[a] *Metaphylaxis is the group administration of an antimicrobial to a population at risk for disease before overt clinical disease is apparent in the entire group.*
[b] *Treatment is the administration of an antimicrobial to an individual animal diagnosed with clinical disease.*

TABLE 7 | In-feed antimicrobial use by indication and class of medically important antimicrobial drug (AMD)[a], organized by category of importance to human medicine[b] and expressed in total number of animal daily doses (nADD).

			Indication			
			Histophilosis	Liver abscesses	Other	Total
AMD Class		AMD	nADD No. (% of use of specific AMD class, % of use for specified indication)			
II	MAC	Tylosin	0 (0, 0)	1,903,454 (100.0, 11.1)	0 (0, 0)	1,903,454
III	TET	Chlortetracycline	11,531,483 (43.3, 100.0)	15,052,190 (56.5, 88.1)	37,815 (0.1, 49.6)	26,621,488
		Oxytetracycline	0 (0, 0)	127,187 (76.8, 0.7)	38,468 (23.2, 50.4)	165,655
		Total	11,531,483	17,082,831	76,283	28,690,597

The nADD are presented in the left part of the cell, and the % of the use of the specific AMD class and the % of use for the specified indication are presented in the right part of the cell separated by a comma. Percentages of use may not add to 100% due to rounding.
[a] *MAC, macrolides; TET, tetracyclines.*
[b] *Roman numerals I to III designate categories of importance to human medicine as designated by the Canadian Veterinary Drugs Directorate.*

TABLE 8 | Number and percentage of cattle (placed 2008–2012) exposed to antimicrobial drugs in-feed, individually dosed, and associated with hormone implants.

		Type of antimicrobial exposure[b]		
	All cattle	In feed	Individually dosed	With implant
OVERALL, NO. (% OF TOTAL CATTLE)				
	2,615,082 (100)	2,527,316 (97)	1,910,825 (73)	544,790 (21)
AGE AT ARRIVAL, NO. (% OF TOTAL CATTLE, % OF CALVES OR YEARLINGS)				
Calf	1,180,499 (45)	1,151,277 (44, 97)	1,060,838 (41, 90)	254,633 (10, 22)
Yearling	1,434,583 (55)	1,376,039 (53, 96)	849,987 (33, 59)	290,157 (11, 20)
SEX, NO. (% OF TOTAL CATTLE, % OF SPECIFIC ROUTE OF EXPOSURE)				
Male	1,643,528 (63)	1,607,049 (61, 98)	1,291,246 (49, 79)	327,661 (13, 20)
Female	971,554 (37)	920,267 (35, 95)	619,579 (24, 64)	217,129 (8, 22)
SEASON OF ARRIVAL, NO. (% OF TOTAL CATTLE, % OF SPECIFIC ROUTE OF EXPOSURE)				
Cold	1,616,686 (62)	1,565,713 (60, 97)	1,314,050 (50, 81)	357,547 (14, 22)
Warm	998,396 (38)	961,603 (37, 96)	596,775 (23, 60)	187,243 (7, 19)
BRD[a] RISK CATEGORY, NO. (% OF TOTAL CATTLE, % OF SPECIFIC ROUTE OF EXPOSURE)				
High	1,021,639 (39)	1,005,810 (38, 98)	971,146 (37, 95)	248,377 (9, 24)
Low	1,593,443 (61)	1,521,506 (58, 95)	939,679 (36, 59)	296,413 (11, 19)

The number of exposed cattle is presented in the left part of the cell.
For the overall number, the percentage of overall cattle this number represents is presented in the right part of the cell in parentheses. When cattle are stratified by particular characteristics and type of antimicrobial exposure, first the percentage of total cattle is presented in parentheses, followed by the percentage of cattle with that characteristic. Percentages of use may not add to 100% due to rounding.
[a] *Bovine Respiratory Disease.*
[b] *Individual cattle may be exposed to antimicrobial drugs via more than one route.*

TABLE 9 | Number and percentage, with 95% confidence interval (CI), of cattle (placed 2008-2012) exposed to individually dosed AMD for different indications by placement cohort (PC)[a].

	Placement cohort				
	1 (*n* = 717,176)	2 (*n* = 670,066)	3 (*n* = 648,916)	4 (*n* = 578,924)	Total (*n* = 2,615,082)
CATTLE EXPOSED, NO. (%OF PC; 95% CI)					
Metaphylaxis[b] or Treatment[c] for BRD[d]	528,117 (73.6; 73.54–73.74)	492,271 (73.5; 73.36–73.57)	452,987 (69.8; 69.70–69.92)	408,678 (70.6; 70.48–70. 71)	1,882,053 (72.0;71.91–72.02)
Metaphylaxis for BRD	513,897 (71.7; 71.55–71.76)	481,549 (71.9; 71.76–71.97)	440,938 (67.9; 67.84–68.06)	398,082 (68.8; 68.64–68.88)	1,834,466 (70.1;70.09–70.20)
Treatment for BRD	48,220 (6.7; 6.67–6.78)	39,278 (5.9; 5.81–5.92)	34,652 (5.3; 5.29–5.39)	31,099 (5.4; 5.31–5.43)	153,249 (5.9; 5.83–5.89)
Treatment for Reason other than BRD	33,359 (4.7; 4.60–4.70)	26,030 (3.9; 3.84–3.93)	24,261 (3.7; 3.69–3.78)	28,722 (5.0; 4.91–5.02)	112,372 (4.3; 4.27–4.32)

The number of exposed cattle is presented in the left side of the cell. On the right side of the cell, the estimate of the percentage of cattle in the placement cohort this number represents is presented with the 95% CI to the right after a semi-colon.

[a]*Placement cohort comprised of cattle placed in the feedlot between I November and 31 October of consecutive years.*

[b]*Metaphylaxis is the group administration of an antimicrobial to a population at risk for disease before overt clinical disease is apparent in the entire group.*

[c]*Treatment is administration of an antimicrobial to an individual animal diagnosed with clinical disease.*

[d]*Bovine Respiratory Disease.*

classifies ceftiofur, fluoroquinolones, and macrolides all as highest priority—critically important antimicrobials (HP-CIA) (33), further underscoring the importance of stewardship in these classes of AMD supported by surveillance data like those presented in the current study.

This study also emphasized the importance of transparency in clarifying the metric used to report AMU, particularly in livestock, since metrics have not been well-standardized (30, 34). **Figures 2A,B** demonstrated the contrast of the gAMD and nADD metric in the specific case of macrolides, which have a relatively low mg/kg dosage and a relatively long duration of effect. In this context, employment of the gAMD metric would result in the interpretation that less macrolides were used in the population than if the nADD metric was used. If only AMU in the same class of antimicrobials was being evaluated in the same production class of animals, the choice of metric would be immaterial. However, if the intention is to compare AMU across classes of AMD (for example, comparing macrolide to tetracycline use) or among different sized animals, the gAMD metric is problematic. Furthermore, because many of the more medically important category AMD, such as cephalosporins and macrolides, have lower dosage per kg rates, emphasis on mg/kg reduction targets could inadvertently discourage appropriate stewardship (35). In summary, weight of AMD can be a useful, intuitive metric if comparing AMU of the same AMD type. If a denominator of biomass or number of animals at risk of exposure is employed, it can potentially be used for comparisons among populations or even across species, but limitations of the metrics must be recognized and transparently reported. It is particularly important in this context that the animal weight used to calculate biomass at risk of exposure is appropriate and standardized among different populations (36). Issues regarding consequences of choice of metrics are covered in more detail in the accompanying paper (37).

Consistent with the primary importance of BRD as a health concern in fed cattle (9), about 40% of in-feed AMU and the majority of individually dosed AMU was related to BRD. The preponderance of individually dosed AMU was for metaphylaxis, and the assessed risk level of the cattle for BRD appeared to have a marked influence on AMD choice for metaphylaxis, with HR cattle far more likely to be exposed to a macrolide for metaphylaxis and less likely to be exposed to a tetracycline than LR cattle, and vice versa for tetracyclines. This is not surprising as macrolides have previously been shown to be highly effective AMD for the prevention of BRD in cattle populations at HR of developing BRD, which influences protocols for AMU (15). It should be noted that because of the relatively larger numbers of LR cattle placed in the studied feedlots compared to HR cattle, tetracyclines were still the most-used AMD on an nADD basis for BRD metaphylaxis. The assessed BRD risk of the cattle also had a less marked influence on AMD choices for BRD treatment. If protocols were unchanged, decreasing the proportions of HR cattle admitted to feedlots could reduce category II AMD (macrolide) and increase category III (tetracycline) use for metaphylaxis, which could be favorable from an AMD stewardship standpoint. However, some factors likely influencing the designation of cattle as HR for BRD, such

TABLE 10 | Medically important antimicrobial use (all routes) by indication and class/type of antimicrobial drug (AMD)[a], organized by category of importance to human medicine[b], and expressed in number of animal daily doses (nADD).

		Placement cohort (PC)[c]				
		1	2	3	4	Total
CATTLE AT RISK, NO.						
		717,176	670,066	648,916	578,924	2,615,082
CATTLE EXPOSED TO AMD PARENTERALLY, NO. (% OF CATTLE AT RISK IN PC)						
		537,599 (75)	498,618 (74)	457,940 (71)	416,668 (72)	1,910,825 (73)
CATTLE EXPOSED TO AMD IN FEED, NO. (% OF CATTLE AT RISK IN PC)						
		694,890 (97)	655,100 (98)	624,899 (96)	552,427 (95)	2,527,316 (97)
nadd, NO. (NO./100,000 CATTLE AT RISK)						
I	FQ[id]	17,512 (2,442)	10,197 (1,522)	8,356 (1,288)	8,382 (1,448)	44,447 (1,700)
	CEPH[id]	65,512 (9,135)	53,934 (8,049)	47,881 (7,379)	53,366 (9,218)	220,693 (8,439)
II	MAC[id]	587,157 (81,871)	505,405 (75,426)	539,728 (83,174)	489,167 (84,496)	2,121,457 (81,124)
	MAC[if]	524,514 (73,136)	492,342 (73,477)	462,974 (71,346)	423,624 (73,174)	1,903,454 (72,788)
	PEN[id]	687 (96)	492 (73)	415 (64)	55 (9)	1,649 (63)
	TMS[id]	30,402 (4,239)	28,785 (4,296)	27,852 (4,292)	28,712 (4,960)	115,751 (4,426)
III	TET[id]	942,109 (131,364)	857,577 (127,984)	688,886 (106,160)	584,480 (100,960)	3,073,052 (117,513)
	TET[if]	7,776,118 (1,084,269)	7,047,227 (1,051,721)	6,642,928 (1,023,696)	5,320,871 (919,097)	26,787,144 (1,024,333)
	PHEN[id]	36,334 (5,066)	75,732 (11,302)	68,938 (10,624)	60,528 (10,455)	241,532 (9,236)
	SULF[id]	497 (69)	396 (59)	245 (38)	279 (48)	1,417 (54)
	TOTAL	9,980,842 (1,391,686)	9,072,087 (1,353,909)	8,488,203 (1,308,059)	6,969,464 (1,203,865)	34,510,596 (1,319,676)

Where number of cattle are presented, the number of cattle is presented to the left the cell with the % of cattle this number represents in the placement cohort in parentheses to the right of the cell. Where nADD are presented, the nADD is presented in the left part of the cell and the nADD/100,000 cattle is presented in the right part of the cell in parentheses.
[a]FQ, fluoroquinolones; CEPH, 3rd generation cephalosporins; MAC, macrolides; PEN, penicillin; TMS, trimethoprim-sulfamethoxazole; TET, tetracyclines; PHEN, phenicols; SULF, sulfonamides. The superscript "id" indicates individually dosed and the superscript "if" indicates in feed.
[b]Roman numerals I to III designate category of importance to human medicine as designated by the Canadian Veterinary Drugs Directorate.
[c]Placement cohort comprised of cattle placed in the feedlot between 1 November and 31 October of consecutive years.

TABLE 11 | In-feed antimicrobial use, by placement cohort[a], antimicrobial class[b], and indication expressed in number of animal daily doses (nADD) and nADD/100,000 cattle at risk, cattle placed 2008–2012.

	Placement cohort			
	1	2	3	4
Cattle at risk	***n* = 717,176**	***n* = 670,066**	***n* = 648,916**	***n* = 578,924**
nADD, No. (No./100,000 cattle at risk)				
HISTOPHILOSIS THERAPY				
CTC (1 g/head)	465,982 (64,975)	406,967 (60,735)	497,428 (76,655)	440,371 (76,067)
CTC (4–7 g/head)	3,125,013 (435,739)	2,826,681 (421,851)	2,321,111 (357,691)	1,447,931 (250,107)
Total	3,590,995 (500,713)	3,233,648 (482,586)	2,818,539 (434,346)	1,888,302 (326,174)
LIVER ABSCESSES				
CTC (35 mg/kg DM)	4,131,126 (576,027)	3,748,300 (559,393)	3,763,010 (579,892)	3,409,754 (588,981)
OTC (11 mg/kgDM)	40,071 (5,587)	31,987 (4,774)	39,754 (6,126)	15,375 (2,656)
TY (11 mg/kg DM)	524,514 (73,136)	492,342 (73,477)	462,974 (71,346)	423,624 (73,174)
Total	4,695,711 (654,750)	4,272,629 (637,643)	4,265,738 (657,364)	3,848,753 (664,811)
OTHER USE (EXAMPLES: PODODERMATITIS, KERATOCONJUNCTIVITIS)				
CTC (1 g/head)	5,044 (703)	21,643 (3,230)	6,938 (1,069)	4,191 (724)
OTC (1–3 g/head)	8,881 (1,238)	11,650 (1,739)	14,687 (2,263)	3,250 (561)
Total	13,925 (1,942)	33,293 (4,969)	21,625 (3,332)	7,441 (1,285)

The nADD is presented to the left of the cell, with the nADD/100,000 presented to the right of the cell in parentheses.
[a]Placement cohort comprised of cattle placed in the feedlot between 1 November and 31 October of consecutive years.
[b]CTC, chlortetracycline; OTC, oxytetracycline; TY, tylosin; DM, dry matter.

as placement of cattle on feedlots during cold winter weather, would be difficult to modify given that one of the underlying reasons for placing the animals on the feedlot during this season is lack of winter pasture. Further, complicated questions about the economics of conditioning animals to reduce BRD risk (e.g., pre-feedlot vaccination, additional "backgrounding" time) have not, as of yet, been addressed within the current farm to slaughter beef industry continuum.

TABLE 12 | In-feed ionophore use, by placement cohort[a] and ionophore type[b], expressed in number of animal daily doses (nADD), cattle placed 2008–2012.

	Placement cohort				
	1	2	3	4	Total
Cattle at risk	***n* = 717,176**	***n* = 670,066**	***n* = 648,916**	***n* = 578,924**	***n* = 2,615,082**
nADD, NO. (NO./100,000 CATTLE AT RISK)					
MON	69,129,832 (9,639,173)	64,538,569 (9,631,673)	63,584,520 (9,798,575)	57,007,350 (9,847,122)	254,260,271 (9,722,841)
LAS	0	0	0	92,337 (15,950)	92,337 (3,531)
ALL	69,129,832 (9,639,173)	64,538,569 (9,631,673)	63,584,520 (9,798,575)	57,099,687 (9,863,071)	254,352,608 (9,726,372)

The nADD is presented at the top of the cell with the nADD/100,000 cattle presented at the bottom of the cell in parentheses.
[a]*Placement cohort comprised of cattle placed in the feedlot between 1 November and 31 October of consecutive years.*
[b]*MON, monensin; LAS, lasalocid; ALL, all ionophores.*

Overall use of AMD decreased over time throughout the study. Since in-feed tetracyclines made up the bulk of medically important AMU, the decrease in overall tetracycline use was primarily driven by the in-feed reduction observed for the indication of histophilosis therapy. This observation provided an interesting example of ability to use these AMU data to assess an intervention. Multi-year clinical studies performed by Feedlot Health just prior to the initiation of data collection for this study indicated that targeted parenteral metaphylaxis reduced the need for in-feed chlortetracycline to prevent and control histophilosis in specific populations. Implementation of new protocols drawing from this study likely resulted in the observed reduction of tetracycline use in-feed. Regarding observed trends for parenterally administered drugs, the overall reduction in AMU over time is most likely a result of continued efforts to improve animal health and welfare in a cost-effective manner. These could include changes regarding vaccine use, biosecurity, animal husbandry, detection of sick animals, metaphylaxis and treatment protocols, and risk assessment/assignment algorithms. The slight increase in the amount of individually dosed macrolides seen from PC1 to PC4 may have been a result of bolstered individually-dosed metaphylaxis. One could argue that it is undesirable to increase category II use while category III use was reduced. However, the magnitude of the reduction in tetracyclines (174,539 nADD/100,000 cattle) was much greater than the small increase observed in macrolides (2,625 nADD/100,000), and group exposures were reduced, arguably improving stewardship.

The other protocol alteration reflected in these AMU data was the addition of a newly licensed product combination of florfenicol and flunixin meglumine based on Feedlot Health clinical research (38) in the fall of 2008. The doubling in florfenicol use from PC1 to PC4 is explained by this protocol change and provides another interesting example of how detailed AMU data such as these could be used to assess effects of interventions.

Overall, this study demonstrated the importance of collecting farm level data to provide a comprehensive picture of AMU in the context of indication for use and animal characteristics. While census data were collected in this study, this is not a practical approach for ongoing, sustainable monitoring of AMU in feedlots due to the time and resources required for data retrieval, collation and analysis. Therefore, future research should focus on appropriate sub-sampling methods for representative monitoring of AMU in fed cattle.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The protocol for this project was reviewed and approved by the Feedlot Health Management Services Ltd. Animal Care Committee (a certified holder of a Certificate of Good Animal Practice) and in accordance with standards set by the Canadian Council of Animal Care.

AUTHOR CONTRIBUTIONS

SB performed summarization, analysis, interpretation of data, and drafted the manuscript. SH was the principal investigator of the study and was involved in study planning, AMU data collection, finalization of data compilation and verification, data summarization and interpretation, and manuscript revision. SG and PM were involved in study planning, interpretation of results, and manuscript revision. BW, JW, JS, and CW assisted in collection, compilation, and verification of data. SO contributed to interpretation of the results and participated in manuscript revision. CB was involved in study planning, oversaw final data verification, summarization and interpretation of findings, and participated in manuscript revision.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2019.00329/full#supplementary-material

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Conflict of Interest: CB is part owner and managing partner of Feedlot Health Management Services Ltd. and Southern Alberta Veterinary Services. SH, BW, JW, JS, and CW are employees at Feedlot Health Management Services Ltd., Okotoks, Alberta, Canada. Feedlot Health is a private company that provides expert consultation regarding production and management of feedlot cattle and calf grower calves, including developing veterinary protocols to support animal health. They also conduct in-house and contract research related to dairy calf grower and feedlot production.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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How Input Parameters and Calculation Rules Influence On-Farm Antimicrobial Use Indicators in Animals

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A variety of indicators of antimicrobial use are available in veterinary medicine, their choice should depend on the study objective as none has been recognized as the most appropriate metric. Calculation of indicators of antimicrobial use is based on a number of parameters (e.g., treatment dose or weight at treatment) that can be informed using theoretical (also called "standard") or actual (also called "used") values. Although few studies compare the application of several indicators to the same antimicrobial data, the obtained results lead to apparent discrepancies or contradictions. This study aimed to investigate antimicrobial use at the weaning stage in French pig farms and, more specifically, the impact the sources of information regarding doses, body weight at treatment and treatment length, had on the indicators results. A cross-sectional survey was conducted, and data collected from 70 farms made it possible to calculate four indicators at the weaning stage using different input values. The indicator values did not show significant differences when calculated based on the theoretical dose and length of treatment (as recommended by the summary of product characteristics) or when calculated based on the dose used and treatment length as applied by the farmer. However, all of the indicators showed significant differences when calculated using the standard theoretical weight (15 kg) or actual weight ($P < 0.05$). It appears that if data collection plans cannot be harmonized, clarification of indicator calculations in the literature is needed to allow comparisons between studies.

Keywords: ALEA, antimicrobials, metrics, nCD, nDD, swine, treatment incidence, weaning

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INTRODUCTION

Although numerous infectious diseases have been successfully controlled during the Twentieth century through the use of antimicrobial agents, prevention of antimicrobial resistance is a major and worldwide public health issue today (1, 2). Shared between human and animal medicine, prevention of antimicrobial resistance requires a reduction in antimicrobial use (AMU), which is the main driver for resistance (3–5). Thus, interventions that reduce AMU in food-producing animals can lead to a reduction in the presence of antimicrobial-resistant bacteria in the animal species concerned (6). A similar association is found in human populations (7, 8).

In France, a national plan named Ecoantibio 2012–2017 was enforced, aiming at a decrease in AMU of 25% over a period of 5 years. The plan encompassed 40 measures, including better monitoring of AMU, and antimicrobial resistance and harmonization of the procedure at the European and international scales (Anonymous 2011). A reduction of 37% was achieved during this period, and the new 5-year plan Ecoantibio 2 now aims at consolidating these results (9). However, measuring AMU can be quite complicated. The French National Agency for Veterinary Medicine (Agence Nationale du Médicament Vétérinaire, ANMV) monitors variations in antimicrobial sales by pharmaceutical companies yearly; however, this is not the most accurate source of data, because it considers all animal species together, and some products may be used in multiple species, including species that are not the initial target (10). A more accurate description of AMU is available from field surveys, but these are intermittent (e.g., every 3 years in pigs) (11, 12).

A variety of indicators of AMU are available in veterinary medicine, and the choice of these indicators should depend on the study objective as none has been recognized as the most appropriate metric. An indicator of AMU is defined as the amount of antimicrobials consumed normalized to the size of a population at risk of being treated in a defined period (13). Although few studies compare the application of different indicators to the same antimicrobial data, it appears that the results obtained lead to apparent discrepancies or contradictions (14–18). Not only can different methods of calculation be used for the same indicators, different data sources can also be used for each of the parameters of the corresponding formula. Thus, data can be collected at the drug producer level, the drug prescription level (veterinarian), the expenditure or delivery level or the farm level (11). The choice depends on the objective, the desired precision and the time frame as well as on the financial and human resources available to conduct the study. When calculating an indicator, information concerning the at-risk period can be variable depending on whether only the duration of the physiological status of the treated animal or its entire lifespan on the farm is considered. Likewise, weight at treatment can be estimated from the Average Daily Weight Gain (ADWG), obtained by weighting the animals or considered as equal to the European estimate of the mean weight of treated animals at a given production stage. Antimicrobial dose and treatment length can be defined by the national Summary of Product Characteristics (SPC), retrieved from veterinarian prescriptions, or reported by the farmer.

The objective of the study was (i) to describe AMU at the weaning stage in farrow-to-finish indoor pig farms in southwest France and (ii) to investigate how the choice of information sources impacts indicator results and whether the results vary depending on the indicator of interest.

Abbreviations: ADG, average daily weight gain; AMU, antimicrobial use; BW, body weight; SPC, summary of product characteristics; nDD, number of daily doses per animal; nCD, number of entire treatments per animal; TI, number of entire treatments per day; ALEA, animal level exposure to antibiotics; UDD, used daily dose: dose and treatment length declared by the farmer; ADD, animal daily dose: dose and treatment length defined by SCP.

MATERIALS AND METHODS

Sampling and Data Collection

A list of 803 farrow-to-finish indoor farms present in southwest France (Nouvelle-Aquitaine and Occitanie regions) was obtained from the national database BDPORC. Two hundred and seventy-one premises declared over 40 sows; of these, 269 had either a telephone number or an email address or both. Five farms were selected randomly for the pilot study and then discarded. Of the 155 farms that could eventually be contacted within 4 phone call attempts and that complied with the inclusion criteria above, 84 farmers agreed to participate, resulting in a response rate of 54.2%. The response rates in the two regions were not significantly different (chi-2, P-value = 0.21).

The final sample was reduced to 70 farms due to (i) missing information (number of piglets per litter, 1 farm), (ii) missing name of the used medicine (making it impossible to find the SPC, 9 farms), and (iii) inconsistent reported values for antimicrobial dose, e.g., more than five times the SPC value (5 farms). One farm had two of the listed inconsistencies.

Data from the calendar year 2014 were collected in 2015 using a questionnaire administered in a face-to-face interview. The questions were mainly closed and were organized into 8 sections, of which three are of interest here: (i) general information on the farm, including farm size and farm management during the post-weaning stage; (ii) economic and technical results, including mean piglet weight at weaning and at the end of the weaning stage; and (iii) farm health monitoring information (number of visits per year by a veterinarian or a technician) and antimicrobial treatments administered. The details of antimicrobial treatments administered during 2014 were collected: name of the drug and percentage of active substance, route of administration, number of packages or items used, size/volume of the package or item, dose used, administration frequency, number of days the product was administered, age of the animals at the beginning of the treatment, number of animals targeted by the treatment, and type of usage. The type of usage could be either prophylactic (applied to healthy animals for the prevention of particular diseases), metaphylactic (administration of antimicrobials to animals experiencing any level of bacterial disease before overt disease occurred, with the time of intervention depending on the detection of disease outbreaks in a few animals in the group) or therapeutic (treatment only of animals showing symptoms of a disease). The dataset was therefore based on the active substances used in each treatment, on each farm and could include several active substances for a given farm.

Indicators Calculations

Indicators calculation formula were retrieved from Collineau (19) for nDD (number of daily doses per animal), nCD (number of entire treatment per animal), TI (number of entire treatments per day), and ALEA (Animal Level Exposure to Antibiotics). The formulas were implemented in Excel (**Figure 1**).

We used the following notations:

- nDD_{PW}: number of daily doses per animal during the post-weaning period

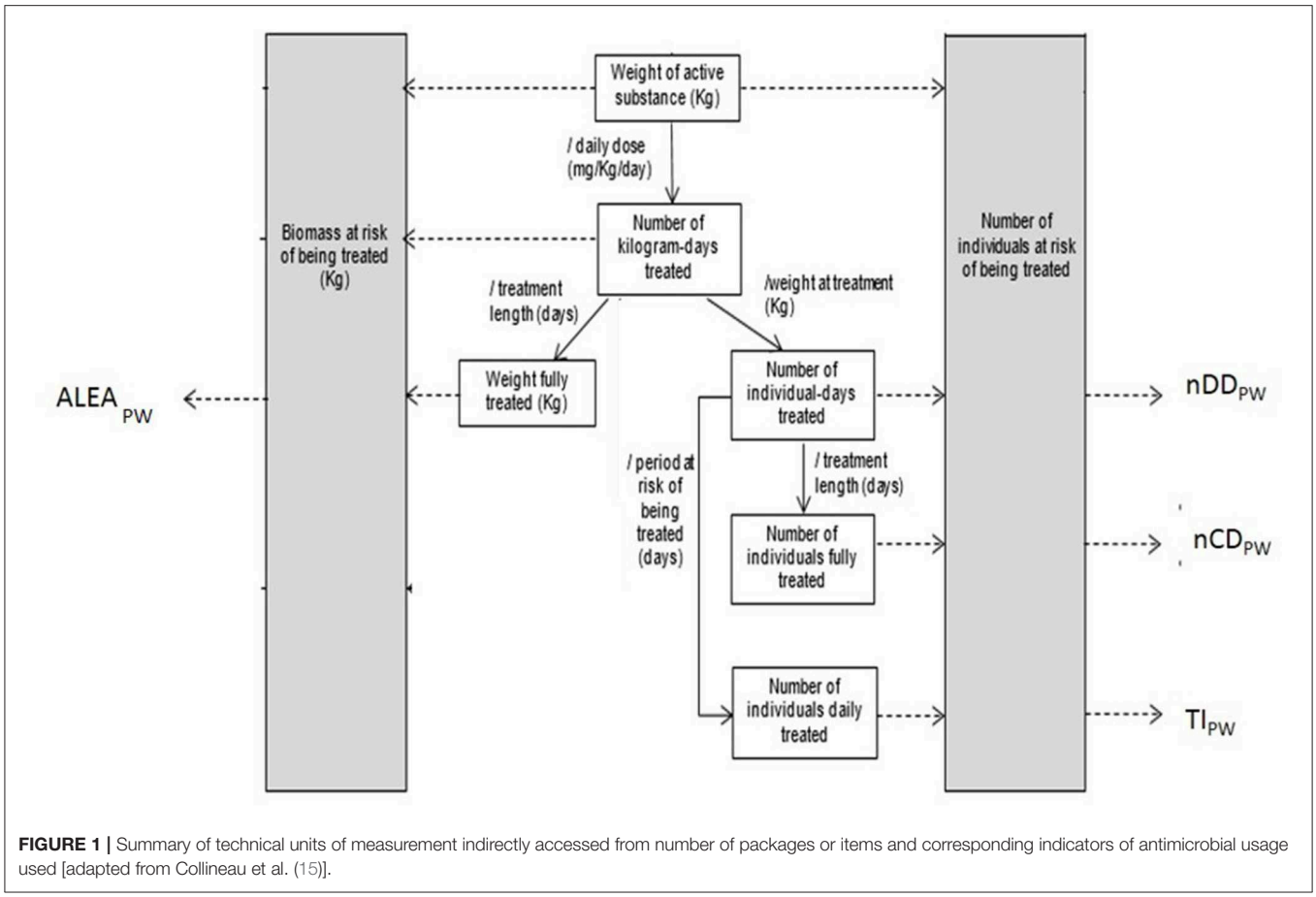

FIGURE 1 | Summary of technical units of measurement indirectly accessed from number of packages or items and corresponding indicators of antimicrobial usage used [adapted from Collineau et al. (15)].

$$nDD = \frac{Weight\ of\ active\ substance\ (mg)}{\frac{dose\ \left(mg.kg^{-1}.day^{-1}\right)\ x\ weight\ at\ treatment(kg)}{Number\ of\ individuals\ at\ risk\ of\ being\ treated}}$$

• nCD_{PW}: number of entire treatments per animal during the post-weaning period

$$nCD = \frac{Weight\ of\ active\ substance\ (mg)}{\frac{dose\ \left(mg.kg^{-1}.day^{-1}\right)\ x\ weight\ at\ treatment\ \ (kg)\ x\ treatment\ length\ (day)}{Number\ of\ individuals\ at\ risk\ of\ being\ treated}}$$

• TI_{PW}: number of entire treatments per day in the post-weaning period

$$TI = \frac{Weight\ of\ active\ substance\ (mg)}{\frac{dose\ \left(mg.kg^{-1}.day^{-1}\right)\ x\ weight\ at\ treatment\ \ (kg)\ x\ period\ at\ risk\ of\ being\ treated(day)}{Number\ of\ individuals\ at\ risk\ of\ being\ treated}}$$

• $ALEA_{PW}$: Animal Level Exposure to Antibiotics during the post-weaning period

$$ALEA = \frac{Weight\ of\ active\ substance\ (mg)}{\frac{dose\ \left(mg.kg^{-1}.day^{-1}\right)\ x\ treatment\ length\ (day)}{Biomass\ at\ risk\ of\ being\ treated\ (kg)}}$$

Indicators were calculated for each of the 70 farms, for the post-weaning period only. The period during which the animals were at risk of being treated was considered to be equal to the duration of the post-weaning period, and the number of individuals at risk of being treated was considered to be equal to the number of post-weaning piglets produced per year (here 2014). The biomass at risk of being treated was calculated as the number of post-weaning piglets produced per year multiplied by the weight of the piglets at the end of the weaning stage. The weight of piglets at treatment used in the calculation was either obtained from the questionnaires (noted as BW, body weight) or the standard theoretical weight was used (noted as 15 kg, mean European value of 15 kg) (20). Similarly, the dose administered and the number of days of treatment were either the values reported by farmers in the questionnaires (called UDD,

Used Daily Dose) or the SPC values (called ADD, Animal Daily Dose). For example, $nDD_{PW-ADD-BW}$ was the notation used when reporting the number of daily doses per animal during post-weaning that had been calculated using actual weight at treatment, dose (ADD) and length of treatment defined by SPC. When using SPC intervals, values corresponding to therapeutic recommendations, meaning the highest dose and the shortest duration of treatment, were selected.

Statistical Analysis

A general descriptive analysis was performed using R 3.4.1 (21). The number and proportion of records below, equal or exceeding the SPC values for either dose or length of treatment and depending on treatment usage (prophylactic, metaphylactic, therapeutic, or all together), administration route (injection, oral), medicated vs. non-medicated feed or individual vs. collective treatment and for colistin were determined. Because of the extreme differences in the doses of different antimicrobial agents (e.g.,: chlorotetracyclin 50 mg/kg and marbofloxacin 2 mg/kg), we calculated relative difference compared to the SPC value. We also considered differences between indicators calculated with (i) ADD vs. UDD and (ii) real weight at treatment (BW) vs. 15 kg (standard European theoretical weight) using non-parametric Wilcoxon paired rank tests. Alpha level for determination of significance was 0.05.

Lorentz curves were built in Microsoft Excel for each indicator calculated using (i) information retrieved from the farmer and (ii) the therapeutic recommendations to investigate whether differences in the calculation could impact farm classification in terms of the proportion of low or heavy users based on the level of AMU. Likewise, Lorentz curves were calculated using only SPC values for dose and treatment length but using (i) BW and alternatively (ii) standard European theoretical weight (15 kg). Lorentz curves represent the cumulative proportion of farms classified, ranging from the lowest antimicrobial user to the highest antimicrobial user (X axis), relative to the total AMU value in the population (of which the cumulative proportion is depicted on the Y axis). Thus, each curve is a graph showing the cumulative proportion of AMU corresponding to x% of the 58 farms having used at least one antimicrobial in 2014 and drawn from the 70 farms included in the survey. The closer a population curve is to the right corner of the graph, the more significant is the proportion of AMU that is contributed by a large sub population of farms that are low users.

TABLE 1 | Sample data from one of the 70 farms included in the survey: this farm used 3 treatments during post-weaning, with 3 different active substances classified in 5 records.

Line 1	Treatment 1	Active substance 1	Benzylpenicillin
Line 2		Active substance 2	Dihydrostreptomycin
Line 3	Treatment 2	Active substance 3	Amoxicillin
Line 4	Treatment 3	Active substance 1	Benzylpenicillin
Line 5		Active substance 2	Dihydrostreptomycin

RESULTS

Differences Between Used and Defined Daily Doses and Treatment Lengths

The size of the 70 farms in the sample ranged from 42 to 1,083 present sows, with a mean of 172. (SD: 1.56). They represented 145 records of active substance, different records possibly corresponding to the same given active substance (**Table 1**). Fifty-eight (82.8%) administered at least one individual or collective antimicrobial treatment at the weaning stage in 2014. Twelve farms (17.1%) that had not used any antimicrobials in 2014 were discarded.

Farmers used from zero to 6 different active substances during post-weaning, with a mean of 1.8 (95% CI: 1.5–2.1) and a median of 2. Nineteen different active substances were used across all farmers surveyed, corresponding to 10 different antimicrobial families with a mean of 1.7 (95% CI: 1.5–2.0), a maximum of 5 and a median of 2 antimicrobial families per farmer. Details on the active substances that were used are given in **Table 2**.

When looking at active substances that were used without distinction between usage type, 16 (11%) doses were over 150% of the SPC value. Regarding real treatment length, 49 records (33.8%) were more than 50% lower than the SPC values and 34 records (23.4%) were more than 150% higher. Sixty-four records corresponded to prophylactic usage, 34 to metaphylactic usage and 47 to therapeutic usage. For prophylactic usage, 13 records (20.3%) were higher than 150% of the SPC dose. Differences in the treatment length appeared more extreme; it was higher

TABLE 2 | List of active substances used by 58 of the 70 farms surveyed.

Family	Active substance	Number of occurrences	Number of farms that used the active substance (% of 58)
B Lactamin	Clavulanic acid	1	1 (1.7%)
	Amoxicillin	16	15 (25.9%)
	Ampicillin	3	3 (5.2%)
	Benzylpenicillin	4	3 (5.2%)
Aminosid	Apramycin	3	3 (5.2%)
	Dihydrostreptomycin	4	3 (5.2%)
	Neomycin	1	1 (1.7%)
	Spectinomycin	10	11 (19%)
Tetracyclin	Chlorotetracyclin	2	1 (1.7%)
	Oxytetracyclin	2	3 (5.2%)
Polymyxine	Colistin	58	50 (86.2%)
Fluoroquinolon	Enrofloxacin	5	5 (8.6%)
	Marbofloxacin	5	5 (8.6%)
Lincosamid	Lincomycin	9	12 (20.7%)
Macrolid	Tilmicosin	3	4 (6.9%)
	Tylosin	10	13 (22.4%)
Diaminopyrimidin	Trimethoprim	2	2 (3.4%)
Sulfamid	Sulfadimethoxin	3	1 (1.7%)
Pleuromutilin	Tiamulin	4	4 (6.9%)

The number of occurrences of each active substance in the dataset is indicated along with the number and proportion of farms that reported using it in 2014.

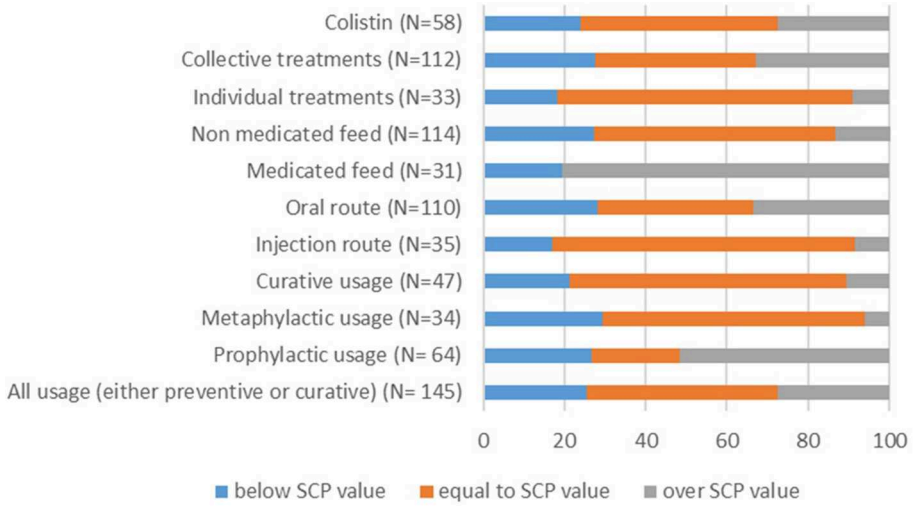

FIGURE 2 | Proportion of records for which used dose (mg/kg/j) is equal to, less than or greater than the standard dose depending on treatment usage (prophylactic, metaphylactic, therapeutic, or all together), administration route (injection, oral), medicated vs. non-medicated feed, individual vs. collective treatment and for colistin. The number of total records per item (N) is indicated in brackets.

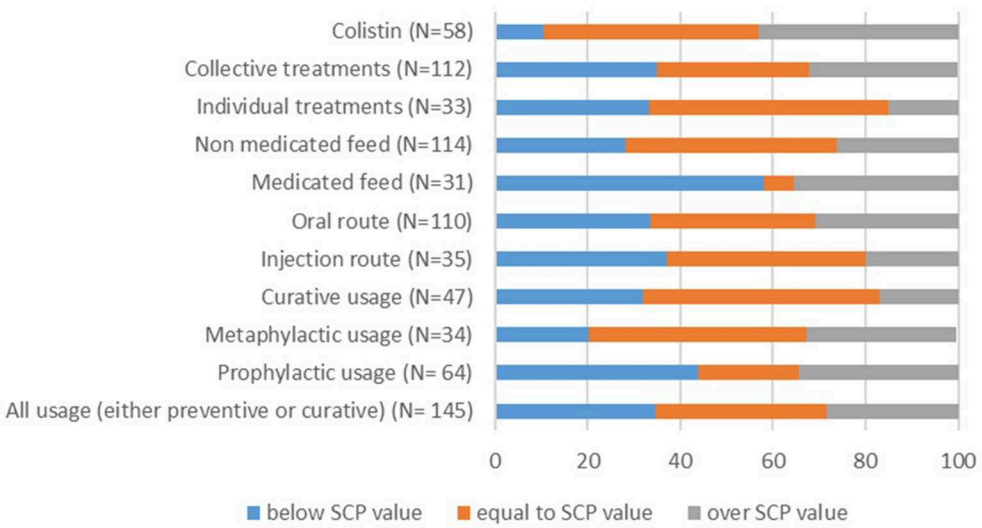

FIGURE 3 | Proportion of records for which real length of treatment (days) is equal to, less than or greater than the standard length of treatment depending on treatment usage (prophylactic, metaphylactic, therapeutic, or all together), administration route (injection, oral), medicated vs. non-medicated feed, individual vs. collective treatment and for colistin. The number of total records per item (N) is indicated in brackets.

than 150% of the SPC value for 18 records (28.1%) and lower than 50% of the SPC value for 27 (42.2%) records. In case of therapeutic treatments, the majority of the records respected the SPC recommendations (**Figures 2**, **3**).

Administration by the oral route was more common than injections (110 records, 75.9% vs. 35 records, 24.1%, respectively). When considering the oral route, 25 records (22.7%) were higher than 125% of the SPC value and 27 (24.5%) involved a treatment length higher than 150% of the SPC value.

There were 31 records of medicated feed vs. 114 records of non-medicated feed drugs. Length of treatment with non-medicated feed drug records was more than 150% higher than the SPC for 26 records (22.8%) and at least 50% shorter for 32 records (28.1%).

Finally, 33 records (22.8%) referred to individual piglet treatments, and 112 records (77.2%) referred to entire batch treatments. Individual treatments appeared to conform more closely to the SPC recommendations. On the other hand, doses used in group treatments were lower than 50% of the SPC value in 27 records (24.1%) and higher than 125% of the SPC value in 25 records (22.3%). In addition, 38 (33.9%) treatment lengths were lower than 50% of the

TABLE 3 | Main description of the calculated indicators of antimicrobial use (minimum, first quartile, median, third quartile, maximum, mean, standard deviation) using different information sources.

Indicator	Min	1st quart	Med	3rd quart	Max	Mean	Sd
$nDD_{PW-ADD-BW}$	0.084	4.48	13.44	23.68	65.95	16.30	14.77
$nDD_{PW-UDD-BW}$	0.084	2.94	12.66	22.53	89.08	18.51	21.14
$nDD_{PW-ADD-15kg}$	0.056	2.55	7.00	15.77	49.19	11.47	12.50
$nCD_{PW-ADD-BW}$	0.042	1.14	2.50	4.06	10.86	3.02	2.50
$nCD_{PW-UDD-BW}$	0.033	0.90	2.76	4.37	21.09	3.45	3.91
$nCD_{PW-ADD-15kg}$	0.022	0.73	1.60	2.91	13.20	2.28	2.66
$TI_{PW-ADD-BW}$	0.003	0.08	0.35	0.45	1.37	0.34	0.33
$TI_{PW-UDD-BW}$	0.003	0.06	0.23	0.48	1.81	0.40	0.47
$TI_{PW-ADD-15kg}$	0.002	0.05	0.12	0.30	1.03	0.24	0.27
$ALEA_{PW-UDD-BW}$	0.019	0.36	0.81	1.46	5.01	1.10	1.11
$ALEA_{PW-ADD-BW}$	0.015	0.29	0.91	1.50	7.07	1.22	1.45

SPC value, and 29 (25.9%) were higher than 150% of the SPC value.

Colistin was found to be the most frequently used active substance (58 records) and was delivered primarily through the using oral route (55 records). Colistin doses were higher than 150% of the SPC value in 4 records (6.9%). Likewise, the treatment length was lower than 50% of the SPC value in 6 records (8.6%) and higher than 150% of the SPC value in 20 records (34.5%). The variations in colistin use were similar to those observed for oral route administration, non-medicated feed, and entire batch treatment because a majority of colistin records were found for these items.

Differences Between Used Weight and Standard Weight at Treatment

In our sample, weaning weight ranged from 5.4 to 9.0 kg, with a mean of 7.6 kg (SE: 0.8). Weight at the end of the post-weaning stage ranged from 15 to 50 kg, with a mean of 29.4 kg (SE: 6.8). Weaning stage duration ranged from 30 to 70 days, with a mean of 47.7 days. There was great variability among farms in these three parameters. The real weight at treatment in our study is equal to the mean standard European weight of 15 kg in 11.03% records ($n = 16$). Animals are lighter at the time of treatment in 78.62% of records ($n = 114$) and heavier in 10.34% of records ($n = 15$).

Impact of Dose and Weight Choice on Indicator Value and Farm Classification

Impact on Indicator Value

Table 3 shows the distribution of the indicators calculated using different values for input parameters. **Table 4** shows the results of the Wilcoxon rank tests.

All of the indicators showed significant differences between calculation with standard weight (15 kg) and actual weight ($P < 0.05$). There was no statistically significant difference between the values of indicators calculated using standard dose and treatment length (SCP) and those calculated using real dose and treatment length as reported by the farmer.

TABLE 4 | Results of the non-parametric Wilcoxon paired rank test on differences between indicators calculated with (i) ADD vs. UDD and (ii) real weight at treatment (BW) vs. 15 kg (standard European theoretical weight).

	UDD vs. ADD	BW vs. 15 kg
nDD_{PW}	0.94	$5.87e^{-9}$
nCD_{PW}	0.68	$7.98e^{-9}$
TI_{PW}	0.86	$5.87e^{-9}$
$ALEA_{PW}$	0.59	

Impact on the Classification of Farms as Heavy or Low Antimicrobial Users

Figure 4 shows the farm cumulative distribution when ADD and UDD are used in the nDD calculation.

Antimicrobial consumption is higher when farm usage (nDD_{UDD}) rather than SPC recommendations (nDD_{ADD}) is considered. Thus, the same amount of nDD is assumed by a smaller proportion of farms when UDD is used in the calculation (∼65%) compared to the result obtained when ADD is used for the same calculus (71% of farms). This difference in indicator result concerns most of the farms for which the two curves do not overlap (∼60%). The Lorentz curves for the other indicators are presented in Appendix 1 (**Supplementary Data Sheet**). Those curves show similar differences between the indicators calculated using UDD and those calculated using ADD, but the gaps between the curves are less important.

Figure 5 shows farm classification when real weight (BW) and standard weight (15 kg) are used in the nDD calculation.

There is no significant difference in the classification of the farms considering nDD_{BW} or nDD_{15kg}. The Lorentz curves for the other indicators (Appendix 1 in **Supplementary Data Sheet**). show a small difference between the nCD_BW curve and the nCD_15 kg curve.

DISCUSSION

For indicator calculations when using SPC intervals, the highest dose and shortest treatment length were selected

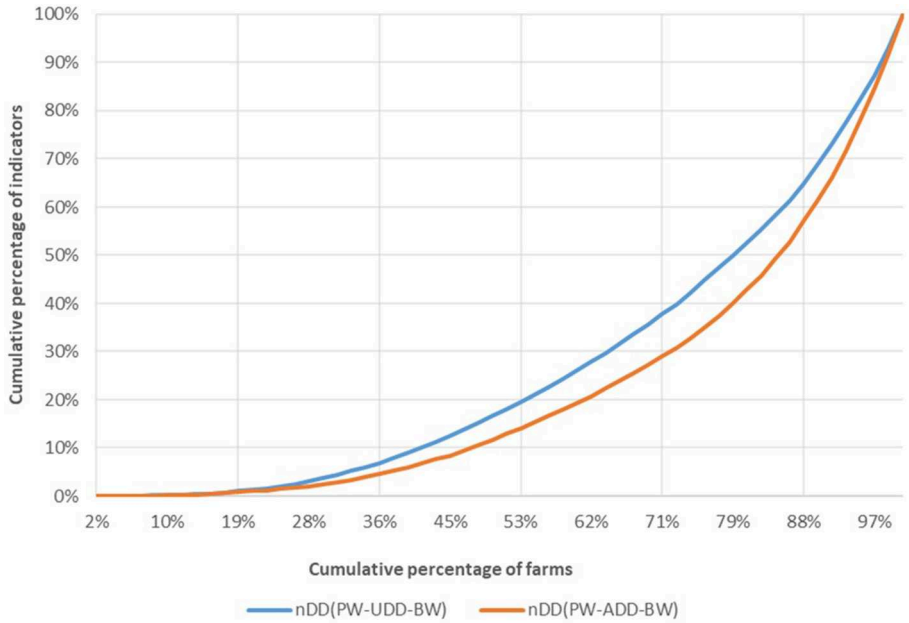

FIGURE 4 | Lorentz curve: comparison between indicators calculated with ADD and UDD, indicator nDD.

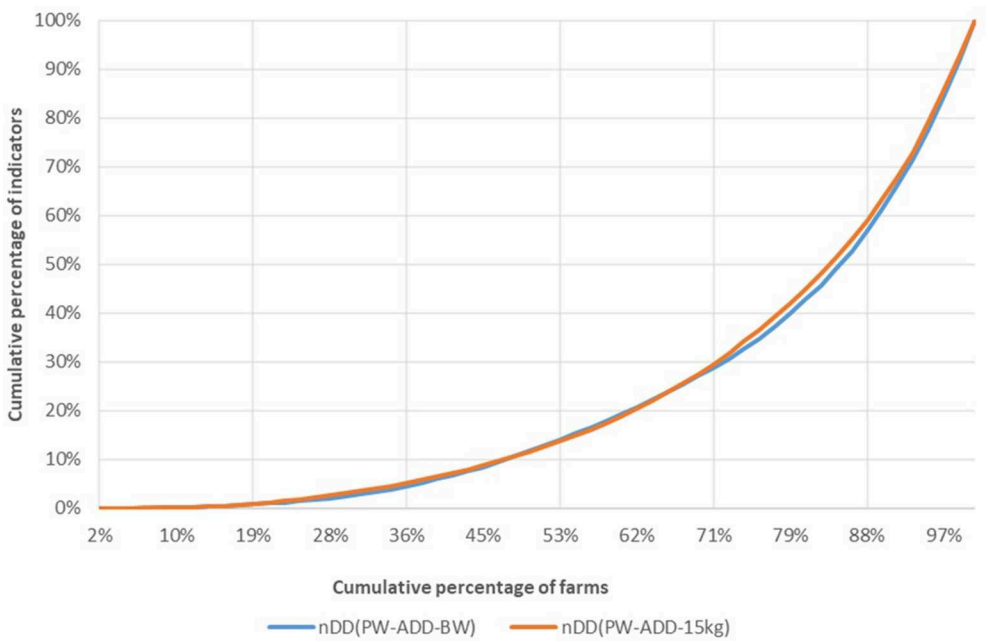

FIGURE 5 | Comparison between indicators of antimicrobial usage calculated based on real weight (BW) and on European theoretical standard weight (15 kg), indicator nDD.

to maintain consistency with therapeutic recommendations but implies that present results include less compliance with SPC recommendations by farmers based upon veterinary prescription compared to field practices. Indeed, this choice influences the differences between UDD and ADD significantly and thus differences may not be regarded as a sign of "UDD was not correct." The objective of the study was to analyze how data selection may influence indicator values and not to analyse compliance with SPC recommendations.

The size of the study sample (70 farms, 145 records) was limited by the exclusion criteria. However, the sample

included farms that showed heterogeneity in size, dose, length of antimicrobial treatment applied and weight of animals. Differences in all indicators were found when calculations based on real weights and standard weights were compared, but no significant differences were found when calculations based on doses were compared. Results cannot be regarded to be representative, although specific reasons for non-representatively could not be identified. Moreover, it is not easy to obtain real and accurate information. Some farms were excluded from this study due to misreported or missing information, although all treatments are compulsorily recorded by the farmer in the farm logs as required by the EU and by national regulations (22). Therefore, a research team must choose between data that are closer to reality and data that are easier to obtain. In the short term, new tools such as GVET that offer farmers a platform for electronic recording should help (23).

The $ALEA_{PW}$ is calculated based on a "biomass at risk of being treated" (**Figure 1**), and we did not analyse the impact of the choice of data for this variable. The weight chosen for the calculation of biomass can be the mean weight of piglets at weaning, the mean weight of piglets at the start of medication or the mean weight of an adult pig.

There was more non-compliance with the recommended length of treatment (72.4%) than with the recommended daily dose (53.1%). According to the Lorentz curves, the nDD is the indicator that shows the greatest difference in farm classification when used dose vs. SPC dose is considered, despite the fact that all of the indicators are influenced by the choice of used or SPC dose, as illustrated by the fact the curves do not completely overlap. We observed that the impact concerns most of the farms, but excludes those with extreme classifications (low or heavy users). The use of ADD for calculation leads to a lower result, and thereby to an underestimation of AMU.

The choice of real or European standard theoretical weight at treatment has a smaller influence on the final classification than the choice of dose and length of treatment. The nCD was the most influenced indicator, and, similar to the findings regarding dose and length of treatment, the choice regarding weight data primarily impacts the middle-user farms rather than the extreme antimicrobial users. Thus, the use of a standard weight for calculation leads to a lower result as well as to an underestimation of AMU. In France, this can be linked to the fact that most treatments are administered at the beginning of the weaning stage (management of diarrhea post-weaning) to piglets weighing less than the ESVAC reference.

Moreover, all countries do not use the same SPC values, these values can be very different, and may question the definition of good therapeutic practices (24). Thus, it could be recommended that real dose, treatment length and real weight be used whenever possible to analyse antimicrobial consumption. Using real values would allow a better description of actual exposure to antimicrobials, although today one would prefer using standard references when aiming at comparisons.

Data from 2014 were collected in southwest France in 2015. Prophylactic usage of antimicrobials, which are still used at high levels in many countries to sustain animal health and welfare was recently banned in feed for farm animals in the EU (25), and these were the treatments for which most discrepancies between ADD and UDD were found in our study.

Colistin use appeared high in this study because the data were collected in 2014, since then, colistin use has drastically decreased in accordance with EMA recommendations (26).

The oral route was more commonly practiced than injection which can be easily explained by the ease of application and the challenge of identifying sick animals in a population, considerations that are involved when managing the effective use of drugs (27). Many pathogens also affect whole groups of pigs, even subclinically, and in such cases the whole group needs to receive antimicrobial medication (28). However, there are issues with administering group medication through the water supply or through feed, mainly with respect to (i) the inter- and intra-individual variability in drinking and feeding behavior and the resulting variability in actual intake of dose; (ii) the risk of AMR damaging the animals' microbiota. In two herds of our sample, the farmers managed to practice injection on whole batches which seems to be a valuable evolution in terms of tackling AMR, although it does not appear as an ideal solution on its own. The use of this method is supported in our study by the fact that injections were practiced in a manner that more closely followed SPC recommendations. Precision livestock farming would be expected to offer opportunities to limit injection-related risks, pain to the animals and costs to the farmer by allowing the early detection of diseased animals.

Our survey did not include questions related to antimicrobial use in sows, piglets during lactation, or pigs during the fattening period. It might have been interesting to investigate whether a low user at the weaning stage was a high user during fattening, for example, which would also have enabled the AMU values found in this study to be compared with those reported in other studies. However, we aimed to maximize the accuracy of data collection by focusing on the weaning stage, which has been identified as the critical period for AMU in pigs. This should enable as a next step the investigation and ordering of risk factors as a basis for proposing practical measures to be implemented in the field to continue decreasing AMU.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comité d'Ethique de l'Ecole Nationale Vétérinaire de Toulouse. The patients/participants provided their written informed consent to participate in this study in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

AW-S designed the study and performed the analysis along with VC and DR. VC drafted the first version of the manuscript. CB participated in designing of the questionnaire and analyzed the pilot study. VC, CB, and MT collected the data in the field. AW-S, VC, LC, AH, CB, MT, and DR contributed significantly to the discussion, read, and approved the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2019.00438/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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