Women in veterinary epidemiology and economics

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Women in veterinary epidemiology and economics

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Editorial: Women in veterinary epidemiology and economics

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Editorial on the Research Topic
Women in veterinary epidemiology and economics

While the number of women graduating from veterinary schools has increased globally over the last few decades, this has not translated into reduced gender bias and inequity in academia and veterinary science research (1). Gender-based discrimination starts at university where women veterinary students are pushed toward "women-majority fields" (e.g., small animal medicine) (2) or where they face discrimination during animal husbandry placements (3). Following graduation, there is clear evidence that gender differences persist in pay and attainment of senior and leadership positions (4). Women's advancement and standing in academic veterinary medicine may in part be influenced by pronounced gender differences in the authorship of veterinary research articles. Women are less likely to be a senior author on a research paper and they are significantly underrepresented in some fields such as surgical and production animal research (5). Gender disparity in professional leadership roles like editorial boards—the median publisher in veterinary sciences had 27.5% editorships belonging to women (6)—can summate by impairing peer recognition and academic advancement.

Our Research Topic aimed to highlight the diversity of work performed across the entire breadth of Veterinary Epidemiology and Economics by teams in which at least 50% of the researchers identified as women.

We start with a veterinary public health study from Thailand carried out by Singhla and Boonyayatra. Their work not only seeks to estimate the prevalence of bovine tuberculosis in slaughtered animals at the Chiang Mai Municipal abattoir, but also to contrast the sensitivity and specificity of the visual meat inspection procedure vs. identification of *M. bovis* by PCR.

A meta-analysis presented by Khanal et al., also from Thailand, looks at the prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) in dairy cattle. MRSA represents a significant zoonotic risk, with a potential of being transmitted not only to dairy farmers but across the dairy supply chain to the wider public. An interesting One Health case study!

Two pieces of research are concerned with management practices to improve animal welfare and herd health in large Hungarian dairy cattle farms. The first by Ózsvári and Ivanyos considers the use of pre- and post-milking teat disinfectants and milking machine cleaning products, and links to udder health, in large commercial Holstein-Friesian farms. The second by Várhidi et al. assesses the use of probiotics in nutrition and herd health management as well as the views and experiences of farm nutrition experts.

The flag of veterinary economics was flown by Jerlström et al.. They use stochastic partial budget analysis to measure the economic impact of two strategies aimed at reducing the prevalence of lung lesions in Swedish pigs.

As well as their important contribution to more traditional veterinary Research Topics, women are pushing boundaries and helping new research fields grow such as outcomes research (Dewsbury et al.). Despite being well-established in human medicine, outcomes research, which entails the application of clinical and population-based methods to optimize healthcare practices and interventions, has only recently started to be explored within the context of animal health and veterinary medicine.

We also want to highlight contributions which are the results of collaborations between female scientists from emerging economies and from leading economies. A team of researchers from Thailand and the US (Boonyayatra et al.) use social networks to describe dairy cattle movements in Northern Thailand and identify highly connected districts which represent key areas for disease transmission, surveillance, and control. A research collaboration spanning Australia and Fiji provide an overview of the long-term Bovine Brucellosis and Tuberculosis Eradication and Control program in Fiji (Argelis Garcia et al.). While important improvements in the program have been noted between 2014 and 2020, enhancements in data capture and harmonization as well as increased farmers compliance are now required to make further progress toward eradication of the diseases. International collaborations could be a positive model to promote gender equality in scholarly authorship, in particular for scientists from regions of the world where men are the most overrepresented academically and professionally.

Two submissions to this Research Topic brought up women's perspective exploring gender balance in the animal production sector and the veterinary profession.

While "women are among the most involved in and served by [farming] co-operative organizations", they are also "the least likely to hold high-ranking and decision-making roles" (7). Hansen and Asmild discuss the structural, cultural, historical, and institutional barriers limiting women's representation on the boards of farmer-owned cooperatives in Denmark. They invite future research to focus on documenting the impact of having more women on boards on the overall performance of cooperatives.

Finally, Stärk et al. challenge the readers to ask themselves why—despite the feminization of veterinary medicine as a profession—so few women either pursue a career leading to, or are successful at securing a leadership role such as being a Chief Veterinary Officer? Several possible explanations to this "leaky pipeline" are put forward but a thorough intentional examination of the field by its practitioners is required to trigger a systemic change in the veterinary medicine work culture, into one where both public and private organization's recruiting and progression policies truly support gender equality and other forms of diversity.

It is important to note that the gender gap in the authorship of veterinary research articles has improved dramatically over the past 20 years (5). Just over two-third (27) of the 39 authors in this Research Topic identified as women. Eighty percent of the first author and of the last author positions were occupied by female scientists. However, it is equally important to observe that more generally significant disparities persist and that the gender gap does vary across and within various geographies. While gender equity in veterinary sciences cannot solely be assessed by looking at research papers, all of us involved in the scientific production and throughout the publication process itself have role to play to increase the visibility of female role models for young women contemplating careers in academia.

Author contributions

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

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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ORIGINAL RESEARCH
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Prevalence, Risk Factors, and Diagnostic Efficacy of Bovine Tuberculosis in Slaughtered Animals at the Chiang Mai Municipal Abattoir, Thailand

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This study aimed to (1) investigate the prevalence of bovine tuberculosis (bTB) in slaughtered animals at the Chiang Mai Municipal abattoir in Chiang Mai, Thailand; (2) identify animal-level risk factors for bTB at the abattoir; and (3) evaluate the performance of techniques for bTB detection at the abattoir. From April 2020 to March 2021, 161 animals registered for slaughter were randomly selected for the study. Animal data including age, sex, species, body condition scores, and origins of the animals were collected. Meat inspection was performed by a trained meat inspector. Tissue samples of the lung, liver, and lymph nodes were collected for histopathological diagnosis and polymerase chain reaction (PCR) detection of Mycobacteria and specifically *Mycobacterium bovis*. The prevalence of bTB during meat inspection and PCR was calculated separately. Animal-level factors affecting bTB were determined using multivariate logistic regression analysis. The performance of meat inspection and PCR was evaluated using a Bayesian approach. The prevalence of bTB was 12.4% (20/161) and 34.8% (56/161) when the disease was diagnosed using meat inspection and PCR, respectively. Buffaloes had a significantly higher risk of being identified as bTB-positive using PCR compared to beef cattle (odds ratio = 2.19; confidence interval = 1.11–4.30). The median of posterior estimates of sensitivity (Se) and specificity (Sp) to detect bTB using meat inspection were 20.8% [95% posterior probability interval (PPI) = 9.1–36.5%] and 87.8% (95% PPI = 79.6–95.4%), respectively. The medians of the posterior estimates of Se and Sp for PCR were 88.6% (95% PPI = 70.5–98.3%) and 94.4% (95% PPI = 84.7–98.8%), respectively. These findings demonstrate that bTB is highly prevalent among slaughtered animals. PCR can be used as an ancillary test for bTB surveillance at abattoirs in Thailand.

Keywords: bovine tuberculosis, prevalence, risk factors, diagnostic efficacy, abattoir, Thailand

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INTRODUCTION

Bovine tuberculosis (bTB), a chronic disease in cattle and buffaloes, is caused by members of the *Mycobacterium tuberculosis* complex (MTBC), including *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium orygis*, *Mycobacterium caprae*, *Mycobacterium microti*, *Mycobacterium pinnipedii*, *Mycobacterium mungi*, and *Mycobacterium suricattae* (1). MTBC can cause tuberculosis in mammals, including domestic and wild animals, and humans *M. bovis* is the most common species reported to cause bTB in domestic cattle and buffaloes. In addition to *M. bovis*, other members of MTBC such as *M. tuberculosis* (2, 3), *M. caprae* (4), *M. orygis* (5), *M. pinnipedii* (6), and *M. microti* (7), have also been reported to cause bTB in cattle. Cattle and buffaloes are susceptible to *M. bovis*. The pathogen can spread to humans primarily through the consumption of milk and meat from infected animals as a zoonotic disease and kills individuals annually (8). Therefore, the disease is a critical public health burden and causes severe economic losses due to impairment of animal health, production losses, costs of eradication programs, and trade restrictions (9). Annual agricultural losses due to bTB have been estimated to be ∼3 billion United States Dollars worldwide (10).

Risk factors associated with bTB outbreaks have been reported at different levels (11). Several studies have previously identified the animal-level risk factors for bTB outbreaks. These factors include age, sex, breed, body weight, average daily gain, immune status, genetic resistance or susceptibility to bTB, vertical and pseudo-vertical transmission, and auto contamination (12–15). The age of animals is a major individual factor that has been identified as a risk in both developed and developing countries (16, 17). A study in Ireland in 1996 found that adult cattle were more likely to be infected with *M. bovis* than younger calves (16). Therefore, it has been hypothesized that exposure duration is positively associated with age.

The bTB eradication and control program is based on the test and slaughter policy and/or surveillance at abattoirs. The abattoir surveillance system is primarily performed by detecting suspected granulomatous lesions during the slaughtering process. The bTB positive animal is traced back to the farm of origin for further bTB investigations through a tuberculin skin test (18–20). The abattoir surveillance system is used as an ancillary method for live animal testing to increase the chance of disease detection in both officially bTB-free (OTF) and non-OTF countries (18). However, the sensitivity (Se) and specificity (Sp) of detecting lesions during meat inspection at abattoir surveillance vary, ranging from 25 to 96% and 22 to 100%, respectively (20–23).

To enhance the performance of a bTB surveillance program at abattoirs, additional assays, such as PCR, can be used to confirm suspected lesions from a meat inspection process (19). PCR has also been implemented for investigations of bTB in slaughtered animals at abattoirs in many countries (24, 25). The performance of PCR has been reported with an Se and Sp of 87.7% (94.3–99.0%) and 93.6% (89.9–96.9%), respectively (19).

Thailand launched a bTB eradication program using a test and slaughter policy for a decade. However, this program has only been implemented in dairy cattle. Beef cattle and buffaloes were not required to participate in the control program. Therefore, this disease has not been eradicated. The bTB is considered a neglected zoonotic disease in Thailand due to its low prevalence in live adult dairy cattle. The bTB surveillance system at abattoirs, which primarily slaughter beef cattle and buffaloes, is rarely performed. Thus, information on bTB at abattoirs, such as prevalence, risk factors, and the efficacy of bTB detection is also scarce. This study aimed to (1) investigate the bTB prevalence in beef cattle and buffaloes at the Chiang Mai Municipal abattoir; (2) identify animal-level risk factors for bTB; and (3) evaluate the performance of meat inspection and PCR for the detection of bTB at abattoirs using a Bayesian approach.

MATERIALS AND METHODS

Study Area and Sample Collection

This cross-sectional study was performed at the Chiang Mai Municipal abattoir, Chiang Mai, Thailand. This Chiang Mai Municipal abattoir is the only abattoir certified for compliance with the Thai agricultural standard on good manufacturing practices (TAS-GMP) for cattle and buffalo abattoir (TAS 9019-2007) in Chiang Mai province. At the abattoir, there was only one single person who was officially trained to be a meat inspector, which is a minimal requirement according to the TAS-GMP standard (26). A sample size required for a population survey was estimated using Epi Info^{TM} version 7.2.3.1 (27) with the following parameters: (1) a population of 800 slaughtered animals in the Chiang Mai Municipal abattoir, (2) expected bTB prevalence of 14% as previously estimated by Singhla et al. (28), and (3) the accepted margin of error of 5%. From the calculation, the minimal required sample size for the current study was 150 animals. Between April 2020 and March 2021, 15 animals registered for slaughtering each week were inspected by a single trained meat inspector, and 3–4 animals per week were randomly selected. Information on slaughtered animals was collected from the abattoir database, including the type of animals, sex, age, body condition score, the origin of animals, and date of slaughter. During the meat inspection process, suspected organs and carcasses with bTB-like lesions were recorded. Tissue samples from the lungs; liver; and lymph nodes such as retropharyngeal, mediastinal, trachea-bronchial, and mesenteric, were aseptically collected from the selected carcasses and sent to the Veterinary Diagnostic Laboratory at the Faculty of Veterinary Medicine, Chiang Mai University for *M. bovis* identification using PCR. The animal study was reviewed and approved by the Animal Use Ethics Committee of the Faculty of Veterinary Medicine, Chiang Mai University (R10/2563).

Meat Inspection

The meat inspection procedure was performed on the intact visceral organs of each of the selected carcasses, particularly the lungs, liver, gastrointestinal tract, and lymph nodes. Each organ was assessed macroscopically by visual inspection, palpation, and incision. All tissue samples of the lymph nodes, lungs, and liver from the carcasses were collected by a single meat inspector of the Chiang Mai Municipal abattoir. During the inspection process, the organs were examined to detect the presence of suspect bTB lesions. The carcasses with tubercle formation, such as an abscess with necrotic focus and caseation and sometimes calcification surrounded by a fibrous capsule, were defined as suspected bTB on meat inspection (29).

DNA Extraction

Tissue samples from each animal were pooled for DNA extraction. Pooled tissue samples were homogenized. A mixture of 300 mg of homogenate with 250 μL of distilled water and 250 μL of lysis buffer was added to a 2-mL tube containing 300 mg of 0.5-mm glass beads and subjected to mechanical disruption at 30 Hz for 20 min. After mechanical lysis of the tissue, DNA was extracted using NucleoSpin® Tissue (MACHEREY-NAGEL, Duren, Germany) according to the manufacturer's instructions.

Genus Identification of *Mycobacterium* spp.

The 16S rRNA gene (1,030 base pairs) of *Mycobacterium* spp. was amplified using PCR from the extracted DNA. Each PCR reaction contained 32.8 μL of distilled water, 0.2 μL of Taq polymerase buffer (5 U/μL), 3 μL of $MgCl_2$ (25 mM), 1 μL of dNTPs (10 mM), 2 μL of 16S rRNA MYCGEN-F primer (5′-AGAGTTTGATCCTGGCTCAG-3′) (10 μM), 2 μL of 16S rRNA MYCGEN-R primer (5′-TGCACACAGGCCACAAGGGA-3′) (10 μM), 5 μL of 10X PCR Buffer, and 5 μL of template DNA. A thermocycler was used for amplification with the following cycling program: 5 min of initial denaturation at 95°C, followed by 35 cycles of 30 s of denaturation at 95°C, 30 s of annealing at 55°C, and 45 s of extension at 72°C, and a final extension at 72°C for 7 min. The PCR products were separated by electrophoresis on a 2% agarose gel and visualized using an ultraviolet illustrator. Positive samples were considered to contain bacteria in the genus *Mycobacterium*.

Detection of MTBC and *M. bovis*

To identify *M. bovis*, the PCR products of the 16S rRNA gene PCR-positive samples were subsequently tested using multiplex PCR for identification of *M. bovis*, as described previously (30). Multiplex PCR was performed with specific primers for the MTBC using TB1 (5′-CCTGCGAGCGTAGGCGTCGG-3′) and TB2 (5′-CTCGTCCAGCGCCGCTTCGG-3′), and *M. bovis-specific* primers using pncATB-1.2 (5′-ATGCGGGCGT TGATCATCGTC-3′) and pncAMB-2 (5′-CGGTGTGC CGGAGAAGCCG-3′). The multiplex PCR was performed by mixing 1 μL of DNA extracts from all mycobacterial reference strains and tissue samples with 24 μL of reaction mixture containing 0.2 pmol μL TB1 and TB2 primers, 0.4 pmol μL pncATB-1.2 and pncAMB-2 primers and other PCR mixtures as described in the previous section. Similar PCR cycles were conducted for amplification as described in the previous section (31–33).

Statistical Analysis

The apparent prevalence of bTB in meat inspections and PCR was calculated separately. The animal-level risk factors were identified using logistic regression analysis. The analyses were divided into two stages and implemented in R version 4.1.0 (34). In the first stage, categorical and continuous variables were primarily screened using univariate logistic regressions via the lme4 package (35). Multicollinearity among predictor variables was evaluated using the chi-square test for categorical variables and Pearson product-moment correlation of continuous variables (cor ≥ 0.5). If multicollinearity was identified ($P < 0.05$, or cor ≥ 0.5), the variable with higher biological plausibility was offered to the next stage.

In the second stage, variables from the previous analysis ($P \leq 0.2$ and without marked multicollinearity among the candidate variables were included in the full multivariate logistic regression for model selection. A stepwise procedure based on the Akaike information criterion (AIC) was performed using the bestglm package (36). The final model was selected from the model with the lowest AIC value. If several candidate models had similarly low AIC values (difference in AIC value < 2), the model with the fewest parameters was preferred as the final model because of the principle of parsimony.

Cohen's kappa analysis was conducted to assess the agreement between the meat inspection and PCR results. The analysis results were categorized into six categories based on kappa values (0–1) into slight (0–0.20), fair (0.21–0.40), moderate (0.41–0.60), substantial (0.61–0.80), and almost perfect (0.80–1.0) agreements (37). Regarding the imperfect characteristics of both meat inspection and PCR from tissue samples, the performance of meat inspection and PCR for the detection of bTB in carcasses and the true prevalence of the disease were analyzed using a Bayesian latent class modeling approach as described by Joseph et al. (38). The Bayesian model assumes that for the k populations, the counts (Y_k) of the different combinations of test results, such as +/+, +/–, –/+, and –/– for two tests follow a multinomial distribution: $Y_k \mid P_{qrk} \sim$ multinomial (n_k, $\{P_{qrk}\}$), where qr is the multinomial cell probability for the two-test outcome combination, and Pqrk is a vector of probabilities of observing the individual combinations of test results. The priors for Se and Sp of both meat inspection and PCR and priors for bTB prevalence rates were derived from previous studies and modeled as beta distributions (19–23, 28). All the priors are listed in **Table 1**. Meat inspection is based on indirect detection of bTB, such as granulomatous lesions in visceral organs, whereas PCR is based on direct detection of the pathogen in clinical samples. Therefore, a Bayesian model for two conditionally independent tests was implemented in a single population to evaluate the Se and Sp of each test and estimate the true disease prevalence. All analyses were performed in JAGS

TABLE 1 | Prior estimates for mode and 95% confidence interval (CI) for sensitivity and specificity values of meat inspection and polymerase chain reaction (PCR) test and prevalence of the disease (%).

Diagnostic tests	Parameters	Mode	95% CI[a]
Meat inspection[b]	Sensitivity	84.0	>35.0
	Specificity	41.0	>12.0
PCR[c]	Sensitivity	87.0	>52.0
	Specificity	97.0	>90.0
Disease prevalence		10.0	<20.0

[a]95% lower or upper credibility interval bound.
[b]Meat inspection.
[c]PCR.

TABLE 2 | Cross-classified test results for bovine tuberculosis in 161 animals from meat inspection and polymerase chain reaction (PCR) tests.

Diagnostic test results		PCR		Total
		Positive	Negative	
Meat inspection	Positive	8	12	20
	Negative	48	93	141
	Total	56	105	161

TABLE 3 | Univariate analysis of variables as $P \leq 0.2$ considered for multivariate analysis.

Variables	Mean ± SE or percentage		Coefficient	*P*-value
	Positive	Negative		
Types of animals			0.78	0.023
- Buffalo	43.8%	56.2%		
- Beef cattle	23.6%	76.4%		
Age of animals	4.9 ± 0.9%	4.5 ± 0.9%	0.33	0.067

TABLE 4 | Final multiple logistic regression models to find factors affecting bovine tuberculosis (bTB) in animals at the Chiang Mai Municipal abattoir, Thailand.

Variables	β[a]	SE[b]	OR[c]	CI[d]	*P*-value
Types of animals (buffalo)	0.78	0.35	2.2	1.1–4.3	0.023

[a]*Regression coefficient.*
[b]*Standard error.*
[c]*Odds ratio.*
[d]*95% confidence interval.*

TABLE 5 | Posterior estimates for median and 95% posterior probability interval (PPI) for sensitivity and specificity of meat inspection and polymerase chain reaction (PCR) test for the diagnosis of bovine tuberculosis, and prevalence of the disease.

Diagnostic tests	Parameters	Median (%)	95% PPI[a] (%)
Meat inspection	Sensitivity	20.8	9.1–36.5
	Specificity	87.8	79.6–95.4
PCR	Sensitivity	88.6	70.5–98.3
	Specificity	94.4	84.7–98.8
Disease prevalence		30.1	22.1–40.4

[a]*95% posterior probability interval.*

4.3.0 via the rjags and R2jags packages from R version 4.1.0 (34, 39, 40). Posterior distributions were computed after 100,000 iterations of the models with the first 10,000 discarded as the burn-in phase.

The model convergence was tested by the Gelman-Rubin diagnostic plot visual inspection using three sample chains with different initial values (41). Model sensitivity was analyzed to evaluate the influence of prior information and the assumption of conditional dependence between meat inspection and PCR on the posterior estimates (42). For these analyses, each prior was replaced by a non-informative uniform 0–1 distribution, and the changes in the model were subsequently considered as appreciable effects of priors on the model (change > 25% of median value). Then, the deviance information criteria (DICs) of the models with and without the covariance term were compared. The model with the lowest DIC was preferred as the final model (41).

RESULTS

Prevalence of bTB at Abattoir

Throughout the study period, 161 animals, including 72 beef cattle and 89 buffaloes, were randomly selected from a total of 780 slaughtered animals. The average age of the sampled animals was 4.7 years. Most of them were females with a body condition score >3 (83.2%). Most beef cattle and buffaloes were from live animal markets located in Chiang Mai province, with percentages of 58.3 and 78.7%, respectively. No clinical signs of any disease were observed in any of the animals before slaughter.

The prevalence of bTB among slaughtered animals was 12.4% (20/161) and 34.8% (56/161) when the disease was diagnosed by meat inspection and PCR, respectively (**Table 2**). From meat inspection, three of 72 beef cattle (4.2%) and 17 of 89 buffaloes (19.1%) were considered positive. Regarding PCR results, 56 carcasses, including 17 of 72 beef cattle (23.6%) and 39 of 89 buffaloes (43.8%), suspected to be positive for the *Mycobacterium* genus were subsequently identified as having the *Mycobacterium tuberculosis* complex. However, PCR products specific for *M. bovis* was not detected from any tissue sample.

Factors Affecting bTB Status of Animals Detecting by PCR

For univariate analysis, two variables, including animal type and animal age, were significantly associated with bTB status ($P \leq 0.2$). No multicollinearity was observed among the variables (**Table 3**). Both variables were included in the multivariate logistic regression analysis of the final model.

Regarding the multivariate logistic regression analysis, the type of animal (beef cattle vs. buffaloes) was the only variable remaining in the final model. The buffaloes had a significantly higher risk of bTB positive for PCR than beef cattle ($P < 0.05$), as shown in **Table 4**.

Performance of Meat Inspection and PCR for the Diagnosis of bTB

The agreement between meat inspection and PCR was slight (Kappa = 0.03). When Bayesian latent class modeling was applied to determine Se and Sp of both techniques, the median of Se estimates for meat inspection was 20.8 with a 95% posterior probability interval (PPI) ranging between 9.1 and 36.5% and much lower than its prior values. Conversely, the estimated Sp of the meat inspection was 87.8% (95% PPI = 79.6–95.4%) and higher than its prior estimate.

The posterior estimates of Se and Sp for PCR were 88.6% (PPI = 70.5–98.3%) and 94.4% (PPI = 84.7–98.8%), respectively, and they were close to their prior values. The posterior estimate for the disease prevalence was higher than the prior estimate with a median value of 30.1% (PPI = 22.1–40.4%), as shown in **Table 5**.

According to the model selection, the conditional dependent model, with a covariance term between meat inspection and PCR, showed a lower DIC value compared to the conditional independent model (22.8 vs. 30.6%, respectively). Thus, the conditional dependent model was selected as the final model. However, the conditional covariance of the model between the meat inspection and PCR was low in both infected and uninfected animals, and the probability intervals of the conditional covariance included 0, which indicated that the correlation between the results of both tests was low.

After the burn-in phase, the model converges properly without autocorrelation. There was no appreciable effect on sensitivity analyses (change > 25% of median value) in the posterior estimates of the Se and Sp of PCR, and the Sp of the meat inspection technique when non-informative distributions were used as priors for any parameter. This result is interpreted as evidence of model robustness. However, a larger change in posterior estimates for the Se of the meat inspection (from 20.8 to 13.5%) was observed when a non-informative prior was used. This finding suggests that the prior of this parameter influences the modeling process.

DISCUSSION

This study reported the prevalence of bTB at the abattoir, as determined by meat inspection and PCR techniques. The prevalence of the disease using PCR was higher than that of meat inspection. The results agree with a study in Ecuador, which reported a higher prevalence of bTB when PCR was used for disease detection compared with meat inspection (17). This finding indicated low sensitivity of the meat inspection because most infected animals are in the early stage of infection or lesions are very small or invisible (43). However, the presence of bTB in animals at the abattoir should be documented, raising concerns regarding zoonotic diseases or food safety in this region.

In the current study, MTBC were reported to be detected from beef cattle and buffaloes slaughtered at the Chiang Mai Municipal abattoir in Thailand. In contrast, a previous study reported all negative results of bTB among swamp buffaloes in northeastern Thailand using the comparative intradermal test, which is the test to detect the MTBC infection in lived animals (44). Our finding suggests that other members of MTBC rather than *M. bovis* might be the cause of bTB among beef cattle and buffaloes in northern Thailand. Several studies demonstrated the infections of different members of MTBC in cattle worldwide, such as *M. caprae* in Spain (4), *M. microti* in France (7), *M. orygis* in Bangladesh (5), and *M. tuberculosis* in several countries such as in China (2), India (45), and South Africa (3). Infections of *M. tuberculosis* in domestic animals have been increasingly concerned as a reverse zoonosis. A study in India reported a higher prevalence of *M. tuberculosis* in cattle than *M. bovis*, which was probably due to the reverse transmission from human to cattle in the endemic areas of human TB (46). In Thailand, a previous study reported *M. tuberculosis* infections in domesticated elephants, suggesting the possibility of a reverse zoonotic transmission from human to animal in the country (47). Findings of the present study did not provide the species identification of MTBC to confirm the existing of reverse zoonoses in beef cattle and buffaloes in Thailand, which should be further investigated in the future study.

The higher prevalence of bTB in buffaloes compared to beef cattle is consistent with previous reports (48, 49). Accordingly, buffaloes posed a higher risk of bTB than beef cattle. This might be explained by the different behaviors of buffaloes and beef cattle. Buffaloes are very social and are likely to aggregate in pastures (48). Moreover, compared to cattle, buffaloes are better adapted to protect themselves from heat than cattle by spending a lot of time wallowing in the mud to reduce heat stress. This could be a potential factor for the spread of the pathogen within the herd (49). Another reason might be the differences in herd management practices between buffalo and beef cattle. In Thailand, buffaloes are typically raised inattentively. For example, buffaloes are generally fed with poor-quality feed and raised without permanent stability. Moreover, genetic differences associated with susceptibility to bTB between buffaloes and beef cattle have never been studied and should be clarified.

According to the TAS-GMP for cattle and buffalo abattoir (TAS 9019-2007), meat inspection by visual inspection, palpation, and incision of visceral organs, is performed in order to ensure the food safety for the consumers. It is not specifically designed for the bTB diagnosis. Biffa et al. demonstrated that the routine abattoir meat inspection had lower efficiency to detect bTB compared to detailed abattoir meat inspection procedures (43). Therefore, only routine abattoir meat inspection is not sufficient for the effective bTB surveillance program. Our findings revealed that the bTB detection results using meat inspection were in poor agreement with those obtained using PCR. A previous study in 2013 also demonstrated a low agreement of bTB diagnosis between meat inspection and bacterial culture (22). Moreover, the conditional dependence of the final model between meat inspection and PCR was low in both infected and non-infected animals, indicating that the test results were independent of each other. The lack of correlation between the test outcomes suggests that their application as parallel tests would help to increase the performance of the surveillance strategy in current bTB eradication programs (50).

The current study reported low Se in meat inspection at the abattoir. This finding agrees with the findings of previous studies (22, 23). A previous study at the municipal abattoir in Ethiopia reported that the Se of meat inspection was only 25% when the bacterial culture was used as a reference test (22). The low Se of meat inspection might be due to the inability to identify visible lesions from early infected animals, the limited time available for each tissue inspection in the processing line, the limitations of inspection facilities (e.g., light intensity, working space and time), and the low experience of meat inspectors (20, 51, 52). Moreover, infections with other members of the MTBC rather than *M.*

bovis may have a lower propensity to cause classical tuberculous lesions in infected cattle. In contrast, the posterior estimates of Sp of meat inspection reported in the present study were higher than those reported in several previous studies, which reported a median Sp ranging between 72 and 77% (20, 22). However, a meta-analysis study in the United Kingdom (UK) and Ireland reported a higher Sp of meat inspection, ranging from 99 to 100% (23). These variations in estimating the Sp of meat inspection may be related to the varied experience of meat inspectors and the difference in the reference tests used to compare with the meat inspection (20). For instance, a study in Ethiopia reported two different Sp estimates of meat inspection to be 72 and 75% when bacterial isolation and PCR were used as the reference tests, respectively (20).

Posterior estimates for Se and Sp of PCR were estimated to be higher than those of meat inspection, which is consistent with previous studies (12, 16). PCR can detect live or dead mycobacteria at all stages of infection. Therefore, the Se of PCR is not influenced by the presence or absence of lesions on animal carcasses (19, 53, 54). However, a study in Spain reported a low Se of PCR (61.1%) when it was implemented in samples without tuberculosis-like lesions (55). Therefore, PCR can be used together with meat inspection to improve the efficiency of bTB detection at abattoirs.

In this study, the posterior estimate of the true prevalence of bTB was quite high. This might be because the test and cull policies have never been implemented for buffaloes and beef cattle in Thailand. Therefore, infected animals are not removed from herds and consequently sent to the abattoirs, leading to an increased chance of bTB detection. Moreover, it has been reported that there are substantial informal trades in live cattle and buffaloes between Thailand and neighboring countries (56). This can increase the risk of bTB outbreaks and the disease prevalence in the country. However, the inferences based on the findings from this study should be cautiously made. The Chiang Mai Municipal abattoir is the only abattoir with a trained meat inspector in Chiang Mai province. To survey the bTB using meat inspection, only animals slaughtered at this particular abattoir were included in the study. Therefore, the estimated prevalence of the selected population might be different from true prevalence estimated from the population of all slaughtered beef cattle and buffaloes in this region due to the selection bias.

CONCLUSION

This study provides the prevalence and risk factors of bTB in animals at the Chiang Mai Municipal abattoir, Thailand. The estimated prevalence of bTB among slaughtered animals was quite high. Buffaloes had a higher risk of bTB infection than beef cattle. The Se and Sp of the PCR were higher than those of meat inspection for the detection of bTB. This finding suggests that PCR can be used as an ancillary test with meat inspection to improve the efficiency of bTB detection in slaughtered animals in an abattoir surveillance system.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by the Animal Use Ethics Committee of the Faculty of Veterinary Medicine, Chiang Mai University (R10/2563).

AUTHOR CONTRIBUTIONS

TS and SB designed the study and wrote the manuscript. TS conducted the laboratory work and analyzed the data. Both authors read and approved the final manuscript.

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Who wants to be a chief veterinary officer (CVO)?–Thoughts on promoting leadership diversity in the public veterinary sector

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Introduction

A Chief Veterinary Officer or CVO is a leadership role within a national Veterinary Service (VS). The title CVO in many countries implies legal responsibility for animal health, animal welfare and food safety for animal-derived food. The term CVO is also used for the delegates representing individual countries in the World Organization for Animal Health (OIE). In the context of this article, we use the term CVO as a placeholder for a leadership role within the veterinary public health community of a country. "Veterinary public health" is understood to cover all aspects of public health, i.e., the science of preventing disease, prolonging life and promoting health through the organized efforts of society (1), where veterinary knowledge is required. We apply a broad definition of public health that includes the more indirect impact of animal health (e.g., economic impact) and animal welfare on human well-being.

Based on correspondence with OIE staff, it becomes clear that only around a quarter of CVOs are women. This is consistent with low numbers of female leaders in other parts of the veterinary medicine sector such as professional organizations (2). This is in stark contrast to the composition of the student body enrolling for the veterinary degree. In most western countries, veterinary medicine has become a feminized profession, i.e., a profession where the gender balance is shifted from male to female (3). While in the 1970's, 16.8% of veterinary graduates in the US were female, this shifted to 44.3% in the 1980's and reaching 65.8% in 1996 (4). In the last survey of the veterinary profession in Europe, 58% of the respondents were female (5).

The question we want to discuss here is the following: Why is it that – out of all these female veterinary students – and at a time in history when women have more access to leadership roles than ever before, do so few pursue a career leading to a leadership role such as being a CVO?

What does it take to become a veterinary public health leader?

In some countries, the only formal requirement to become a CVO (or comparable senior veterinary public health leader) is to have completed a veterinary degree plus a number of years of relevant experience, while in others, the requirements are specified in more detail [e.g., for the European Union, see (6)], listing the knowledge, skills and competencies required to be eligible to act as an Official Veterinarian and thus – ultimately – as a CVO.

Admission to veterinary degree courses tends to be highly competitive in many countries and access is based on merit-focused indicators which favors entry for women. There appears to be a difference in the motivation of female candidates compared to male (7). Over the past 20 years, the veterinary profession has become increasingly feminized (8). Once a profession becomes dominated by females, this is typically associated with the loss of status in society, e.g., in terms of remuneration (2). This then reduces its attractiveness to male candidates, thus resulting in a vicious cycle. In the case of veterinary medicine, increased numbers of female graduates have not translated into more women in academic positions, particularly above associate professor level (9, 10).

At postgraduate level, veterinary public health (VPH) is the specialty field with most relevance to the role of a CVO. VPH is a broad field including aspects of animal health, animal welfare, economics, ethics and legislation. VPH specialists are employed by government veterinary services, by industry, non-government organizations or as consultants. Specialization in VPH can thus be an attractive alternative to clinical specialization for women who plan for a family. Working hours can be more predictable compared to clinical duties including emergency rosters. More importantly, a role in VPH can be perceived as being valuable for society, it is associated with teamwork and with diverse and challenging problems, seen as distinctly different from routine clinical work (Gabrielle Bischopps, personal communication). There is evidence from other science-based disciplines that women tend to be more motivated by the opportunity to make a difference in an area they are passionate about than simply by financial gain or acclaim (11, 12). Based on the list of VPH specialists recognized by the European Board for Veterinary Specialization (EBVS) the female to male ratio among its members is almost equal (75:88; Andreas Wunsch, personal communication). The ratio amongst members in the Australia and New Zealand College of Veterinary Scientists VPH chapter is one woman to three men (17:51) and in the Epidemiology Chapter, one woman to almost two men (94:164) (Allen Petrey and Skye Freuen, personal communication). These numbers do not yet reflect the gender balance of the intake to veterinary schools which - in some cases - can be as high as 4 female students to 1 male or more.

However, becoming a CVO does not only require technical expertise, but it also implies leadership aspiration. While women earn an equal share of university degrees in general, women are under-represented in almost all sectors at senior management levels in most western countries (13). In the US, 40% of veterinary practices are under female management (14). On average, female veterinarians earn less than their male colleagues (5). A recent study by Smith (15) with earning data from veterinary practices in the US showed an unadjusted earning gap of 44%. About half of the earning differences cannot be explained by differences in working hours or other factors known to impact on practice productivity. There are more women in leadership positions in the public sector but even there, they remain underrepresented at senior level (see CVO numbers above).

Moving up the career pathway is linked to an organization's recruiting and progression policies. If you believe that gender discrimination is a thing of the past, you are probably suffering from the so-called "blind-spot bias" (16). This is the tendency of people to see themselves as less prone than others to unconscious influences, i.e., to cognitive bias. While most organizations nowadays acknowledge the value of diversity to foster innovation, creativity, adaptivity and performance, women remain under-represented in management positions. This finding remains even though key leadership traits such as extraversion, openness and conscientiousness are known to be common among women (17). Women are on the one hand, overrepresented in the traits that make up 'transformational leadership' profiles and yet, paradoxically continue to be under-represented at senior leadership level (18). Many managers may reply that women in general lack the ambition to take on more responsibility and that their programmes for promoting women have failed to show impact. One reason for this is that ambitious women are a "norm violation." Assertive women are perceived to be bossy, competent women as cold. No wonder it takes encouragement to aspire to roles associated with such characteristics. This is further aggravated by the fact that female vets are often suffering from the so-called impostor syndrome. This term refers to the tendency to doubt one's abilities despite contrary evidence. In a study conducted by Kogan et al. (19) among veterinarians, they found that feelings of self-doubt and fear of being "discovered as intellectual frauds" was particularly prevalent among female vets with only a few years of experience in practice and residing in New Zealand and the UK.

A lack of leadership ambition may also be due to practical reasons. As state childcare services vary between countries, female vets must often decide between career and family. This is despite the fact that veterinary training is among the most expensive degrees offered in western countries and often heavily subsidized by public funding. It should thus be of economic societal interest to retain as many women as possible in active veterinary employment.

In summary, while in principle, leadership career paths in veterinary public health should be attractive to female candidates given their over-representation at graduate level, the balance appears to shift at some point - resulting in an over-representation of male veterinarians in management roles. Similar observations are reported from the academic sector, a phenomenon referred to as the "leaky pipeline" (20).

Discussion

The reasons for the lack of female leaders in a feminized sector such as veterinary medicine, and specifically in the public veterinary service, are diverse and not fully understood. Yet clearly, diverse teams have advantages, particularly in complex environments. It must therefore be desirable to foster diversity for all organizations and businesses that are serious about achieving their goals.

Based on our professional experience, there are no simple policies to "fix" the situation. From personal experience, we know that it is not enough to appoint one woman into a senior position to trigger a systemic change in work culture. To achieve progress, the first step is to confirm the fundamental value of diversity in leadership styles and career paths. There is not a single best profile for a senior veterinary manager; degrees are important but so is experience. Technical knowledge is essential, but so are skills in communication, negotiation, flexibility and improvisation. The commitment of a diverse leadership team is needed to bring about the change in culture that supports gender equality.

Governments and institutions that have acknowledged the value of gender balanced leadership have a variety of tools available to them to create the opportunities for attaining gender balance (21). These tools include policy development to both recruit and retain female leaders and communication of the success of these initiatives. These policies may include flexible working arrangements, active development of female talent, paid maternity leave, paid and unpaid childcare leave. It has been demonstrated that, over time, gender balanced leadership teams are able to undo previously biased and unequal access to organizational power (21).

Gender awareness training is an important first step which ideally should be started early as part of the veterinary curriculum, and then continued as part of continuing professional development (CPD) in universities and veterinary services. Students should be able to recognize and challenge discourse of limitation and discrimination before these become normalized, internalized and entrenched (22). Gender bias is a reality also in a profession where women are well-represented (23). Stereotypically expected behaviors do disadvantage both men and women. It is interesting to note that the stereotypical representations of leaders generally resemble stereotypes of men rather than stereotypes of women, though this is noted to be decreasing over time (18). It may not be surprising that CVOs are for the most part male given the breadth of experience required to attain the role, the perceptions of tradeoffs needed to be made with family time and the perception of how a CVO should conduct themselves.

Female role models are another important element to allow young women to imagine a future in which both professional aspirations and family planning can be realistically combined (24). Many veterinary schools are offering mentoring programmes where students can meet senior colleagues from different sectors to discuss career options. Even more powerful are female leaders that demonstrate not only the feasibility of career progression but also that this is a desirable path worth pursuing. Such inspiration and encouragement are essential not only for women, but also for men.

Many western countries are facing - or soon will be facing - a shortage of skilled workers. This offers opportunities for applicants; it strengthens their negotiation position. This offers a window of opportunity for women to specify the needs for change to make leadership attractive. The experience of the COVID-19 pandemic has opened new opportunities for flexible working that would not have been considered possible before. Dual income families will benefit from the technological solutions that increase independence of the physical location from which a job can be done.

We do not know enough about what stops women in the public veterinary service from seeking out leadership roles. Gender monitoring - a tool increasingly used by universities to document progress - would help strengthen the evidence base. Clearly, changing perceptions, values and practices is not an easy task; it takes energy, time and resources. Progress and output may not be tangible for some time. As with financial investment, believing in a future return is essential and to realize that others are pursuing the same goal may thus help strengthen our commitment. Here, we have made observations and suggestions based on our experience and on the findings of research in other disciplines. As we continue to mentor and guide a more diverse generation of future veterinary leaders, we look forward to more female veterinary professionals to pursue leadership roles. Key motivators for leadership roles are to gain more control over one's own time and life and to enhance the working lives of others (25). Mentoring can encourage individuals to explore alternative futures; mentoring is both effective and simple. It only takes a mentor that is willing to share advice and a mentee who is interested in learning from someone else's experience. For more systemic change, we need to intentionally examine and articulate the current state and identify and address those factors that limit diversity. External enablers such as leadership training and regular articulation and debate on diversity issues are important drivers of change (25). Thus, we can gradually realize the benefits of a diverse leadership culture, both for individuals and for the wider profession.

Author contributions

The authors jointly developed the concept for this opinion. The first draft was prepared by KS, with RS and MA adding and revising the text. All authors contributed to the article and approved the submitted version.

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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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The use of probiotics in nutrition and herd health management in large Hungarian dairy cattle farms

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In the European Union, there is an increasing need for farm animal nutrition products whose positive effects can replace antibiotics that have been heavily used for decades. Thus, the use of probiotics started to increase in the past few years. In this study, a survey on the practical use of probiotics in Hungarian dairy cattle farms and the related experience of farm nutrition experts was conducted. In addition, we surveyed the state of Hungary for probiotics production and distribution. After direct request *via* phone, nutrition experts responsible for farm feeding programs in 23 large commercial dairy cattle farms and eight managers in different feed distributor companies in Hungary filled out the relevant online questionnaires in 2018. The results show that 69.6% of the surveyed farms used probiotics, most often aiming at the optimization of rumen fermentation, protection against stressors, and supplementation of medical treatments. The most common expected beneficial effects of probiotics were more effective calf raising, larger milk yield, more stable rumen fermentation, and improved stress resistance. None of the respondents experienced any negative effects. In Hungary, five out of eight surveyed feed companies produced probiotic products for cattle, and one just distributed them. Company managers generally thought that farm nutrition experts did not have up-to-date knowledge on probiotics, which is why, these products are often not used in an effective way, and the experts' knowledge should be increased. The own experiments of the distributor companies showed that the probiotic products can improve feed digestibility, the efficacy of calf raising, and the reproductive performance of cows. According to the expectations of distributors, the next generation of probiotic products will be microencapsulated and will contain multiple strains and species of bacteria and prebiotics, too. The goal of the product development is to create probiotics with better effectiveness at a reasonable price, having a complex impact and easier application on the herd level. The study showed that probiotics are already frequently used to prevent diseases in Hungarian dairy herds. However, it can be concluded that there is room for improvement, especially concerning the knowledge transfer about the most effective use of probiotic products.

KEYWORDS
probiotics, dairy, cattle, nutrition, health management

Introduction

In Hungary, the cattle sector accounted for 10.8% of the total gross output of agriculture in 2020 (1), which is why it is of great importance to sustaining cattle farming. The aggregated value of the sales of veterinary antimicrobial agents in 31 European countries in 2020 was 89.0 mg/population correction unit (PCU), but a large difference was observed between the countries with the highest and lowest sales (range from 2.3 mg/PCU to 393.9 mg/PCU and median value of 51.9 mg/PCU). Hungary had 169.9 mg/PCU sales for food-producing animals, which was well-above the European average (2). Due to the ban on the use of antibiotics for growth promotion and disease prevention at the herd level, and the mandatory reduction in antimicrobial usage, there is a growing demand for products in feed for livestock that will have a similar positive effect on production and replace the antibiotics in the European Union (3, 4). This is one of the reasons why probiotics have become the focus of scientific interest more recently and their use on livestock farms started to increase significantly (5); however, there is no available official or scientific data about the probiotics used in Hungary.

Probiotics have been defined by Food and Agriculture Organization (FAO) and World Health Organization (WHO) as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (6). This definition has been widely accepted by the International Scientific Association for Probiotics and Prebiotics (7). This is applicable to both human and animal nutrition; it does not limit the positive health effects on the digestive tract, does not require the alteration of the gut microbiota, but does require the intake of an appropriate amount (although this amount is not precisely defined) and that the microorganism is in a live state at the time of intake (6). Gram-positive *Bacillus, Enterococcus, Lactobacillus, Lactococcus, Pediococcus,* and *Streptococcus* bacterial strains, as well as *Saccharomyces cerevisiae* and *Kluyveromyces* yeasts, are the most used probiotic agents in feed supplements in the European Union (8, 9). Accordingly, it is advantageous if the species is a member of the normal intestinal flora of the target animal, produces antibacterial substances against potential pathogens, is genetically stable, and can adhere to as well as colonize the intestinal mucosa (8, 10).

According to Szabó and Szabó (11), probiotics reduce the pH of the intestinal contents, produce antibacterial substances, reduce the amount of ammonia and toxic amines, increase the non-specific immune responses, improve feed palatability and carbohydrate digestibility, and synthesize amino acids and vitamins. Changes in the microbiota of the digestive tract also affect the health and productivity of the animals; therefore, rumen fermentation can be manipulated to improve production (e.g., improvement of milk yield and quality, live weight gain, and feed conversion ratio of calves) (12). So far, the most significant positive animal health and production effects of probiotic supplementation in ruminants have been achieved during periods of high stress for the animal and its intestinal flora, i.e., during the periods of weaning, the beginning of lactation, and the change to a feed being rich in easily digestible carbohydrates (5). It is important to consider that probiotics are relatively slow acting, for which they also require the creation of favorable conditions for the reproduction of eubiotic microorganisms, respectively, and therefore they can be used mainly as preventive agents (13).

The aims of the study were to survey (1) the practical use of probiotics in large Hungarian dairy cattle farms including the experience and expectations of farm nutrition experts and (2) the probiotics production and distribution of Hungarian feed distributor companies including the managers' opinion about the possible product developments of bovine probiotics.

Materials and methods

The surveys were drafted to define the use of probiotics in commercial Holstein-Friesian farms and the views, and future needs of farm nutrition experts as well as the market experience, opportunities, and forecasts of the Hungarian feed distributor companies regarding probiotics. Two different questionnaires were drafted (Supplementary material), which were reviewed by farm nutrition experts ($n = 3$), dairy cattle veterinary practitioners ($n = 3$), academic professionals ($n = 2$), and veterinary and animal science PhD students ($n = 2$) to receive feedback on content. Based on collected feedback, revisions were made before the questionnaires were sent to potential respondents. This survey used a mixed-method approach, which combines the collection of quantitative and qualitative data. The questionnaires contained several open-ended questions that allowed participants to convey their opinion freely. In the first part of this work, data were collected from farm nutrition experts about the number of cows, milk production and reproductive parameters (lactation milk yield, SCC, average lactation number, and calving interval), own feed production, and feed purchase. We also surveyed the practical use of probiotics in the surveyed farms, including the nutritionists' general knowledge, experience, expectations, and future needs on probiotics. In the second part, we gathered data from managers working in different Hungarian feed distributor companies about their probiotic products and the market trends of these product groups as well as the possible product developments and market niches of bovine probiotics.

Commercial Holstein-Friesian farms were included in the first survey based on the following criteria: computerized on-farm records, participation in milk recording, and willingness to provide data to the authors. The questionnaire was available online in Google Forms from 20 October to 1 November 2018. To access the questionnaire, its link was sent to farm nutrition experts, who had access to farm records and were responsible for the farm feeding program, by personal e-mail after a phone call. A total of 23 Hungarian dairy farms were surveyed, and 91.3% of the nutrition experts (n = 21), who were employed by the farms and each working on one farm, were agricultural engineers and 8.7% were veterinarians (n = 2). Feed production and distribution companies in Hungary were included in the second survey based on the following criteria: distribution and/or production of feedstuffs for ruminants and willingness to provide data to the authors. We also used a questionnaire that was available online in Google Forms from 20 October to 4 November 2018. The managers, who were responsible for feed distribution including probiotics, received the link to access the questionnaire by personal e-mail after a phone call. Responses were received from eight managers working for different feed distribution and production companies.

The participants took part in the survey voluntarily and remained anonymous. Each participant was required to sign a written consent before they began the survey. On average, it took 15–20 mins to fill out the questionnaires. If any questions were raised by the respondents, they were answered and discussed by phone. Each questionnaire has been coded to detect inaccuracies in the data entry. The obtained data were processed in MS Excel (Microsoft Corporation, Redmond, WA, USA).

Results

The use of probiotics in dairy farms

A total of 20,738 cows were kept on the 23 farms, which corresponded to 5.26% of the 393,200 Hungarian dairy cow population as of 1 June 2017 according to the official statistical data (14). The smallest surveyed farm had 200 cows, whereas the largest had 2,100, with an average herd size of 901 cows (milking + dry cows), which was higher than the national average of 407 cows. Albeit, all seven regions of Hungary were covered (min. 1 and max. 7 dairy farms per region were involved), it was a non-representative survey. The lactation milk production corrected for 305 days was 10,513 liters on average (n = 23; min. 9,000 liters; max. 12,900 liters), out of which 95.8% was marketed (n = 23; min. 89.1%; max. 99.5%). The average length of calving interval was 400 days (n = 23; min. 375 days; max. 453 days) and the average number of lactations was 2.5 (n = 23; min. 1.9; max. 5).

The main feedstuffs on the surveyed farms were maize silage, alfalfa haylage, rye haylage, and meadow hay. The nutrition was the same in both winter and summer on 12 farms (52.2%), while on 11 farms (47.8%), the feeding was slightly modified by season. Namely, in the summer period, easily digestible fiber-rich haylage was given and nutritionists used more feed supplements (e.g., yeast) during heat stress. In the survey, 17 out of 23 dairy cattle farms (73.9%) had their own feed mills and none of them purchased all required forage crops for these mills. There were five farms (29.4%) where forage crops were just grown by themselves and there were 12 farms (70.6%), where forage crops were both purchased and grown (n = 17). On the farms with their own feed mills, where forage crops were also purchased and grown, the proportion of purchased crops was, on average, 22% (n = 9; min. 10%; max. 40%). Feed supplements were also produced in 35.3% of farms with feed mills, but none of them fully covered their own feed supplement consumption (n = 17).

Feed supplements were used for various purposes in all 23 farms, most often to optimize rumen fermentation, increase milk production, and prevent metabolic diseases (Figure 1).

Rumen buffers and soluble sugars were used most often to optimize rumen fermentation, while probiotics were used for this purpose in nine farms (39.1%; Figure 2), molasses in three farms (13%), and yeast in two farms (8.7%), respectively.

Of the surveyed 23 farms, 16 used probiotics for some purpose (69.6%), most often to optimize rumen fermentation (Figure 3).

In the farms, probiotics had been used for an average of 6–7 years (n = 16; min. 1; max. 20) by using different administration methods at the same time. Probiotics were mixed into the drinking water or administered by drenching in nine farms (56.3%), were mixed into feed in also nine farms (56.3%), were given as powder in seven farms (43.8%), and as a bolus in four farms (25.0%). One respondent mentioned their use in the form of a paste (3.1%). In 43.8% of the farms, probiotics were only used at the group level, in 18.8% at the individual level, and in 37.5% at both levels, respectively (n = 16). At the animal group level, probiotics were most often used for calves and milking cows (Figure 4).

Probiotics were most often used on the farms around the calving period or in the case of gastrointestinal diseases (e.g., rumen acidosis; Figure 5).

Probiotics were used permanently in 37% and periodically in 63% of the farms. If their use was periodic, the probiotics were most used around the calving period and during calf and heifer rearing, but never used in the dry-cow period (Figure 6).

In two-thirds of the farms (n = 12), the probiotics were expected to increase the efficiency of calf rearing and reduce calf mortality. Half of the respondents (n = 9) expected the use of probiotics to prevent cow diseases, reduce culling rates, and increase milk production (Figure 7).

In 30.4% of the farms (n = 7), the nutrition experts thought that they had enough information about probiotics and their administration. However, 52.2% of the respondents (n = 12) considered the available information to be insufficient and 17.4% (n = 4) could not answer this question. The farm experts evaluated the importance of different procurement factors for probiotics on a 5-point Likert scale (1 = not at all important; 5 = very important). Overall, the most important factors

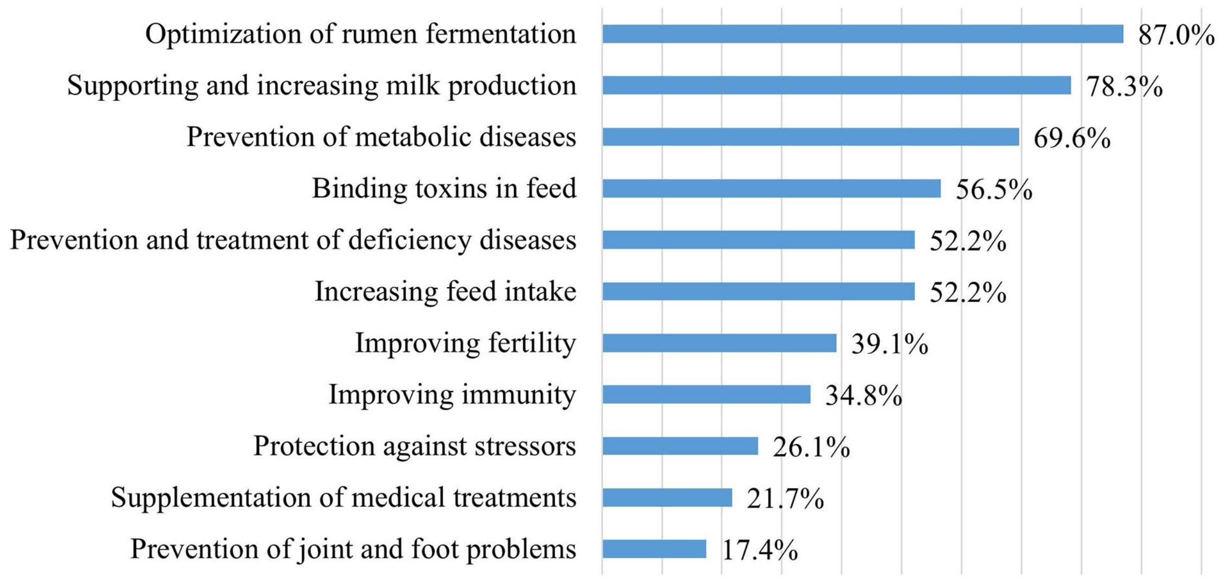

FIGURE 1
The purpose of the use of feed supplements in dairy cattle farms ($n = 23$).

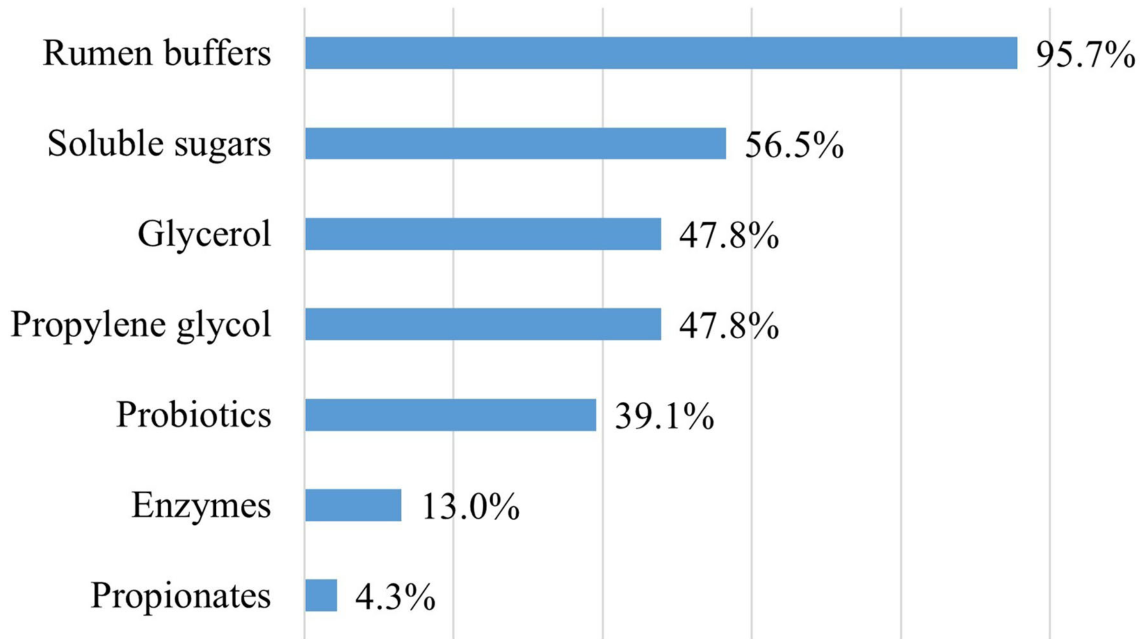

FIGURE 2
Usage of feed additives to optimize rumen fermentation ($n = 23$). Notes: Propionates, which are organic acids, belong to a functional group of additives, and enzymes belong to the enzyme group in the EU animal nutrition.

were the way of administration, the price, and the experience and recommendations of other professionals, while the least important factors were the place of production and the brand name (Figure 8).

To the question "*How many probiotic products do you know?*," 30.4% of the farm nutrition experts ($n = 7$) answered that they were aware of more than five products and 60.9% ($n = 14$) answered 3–5 products. However, one expert (4.3%)

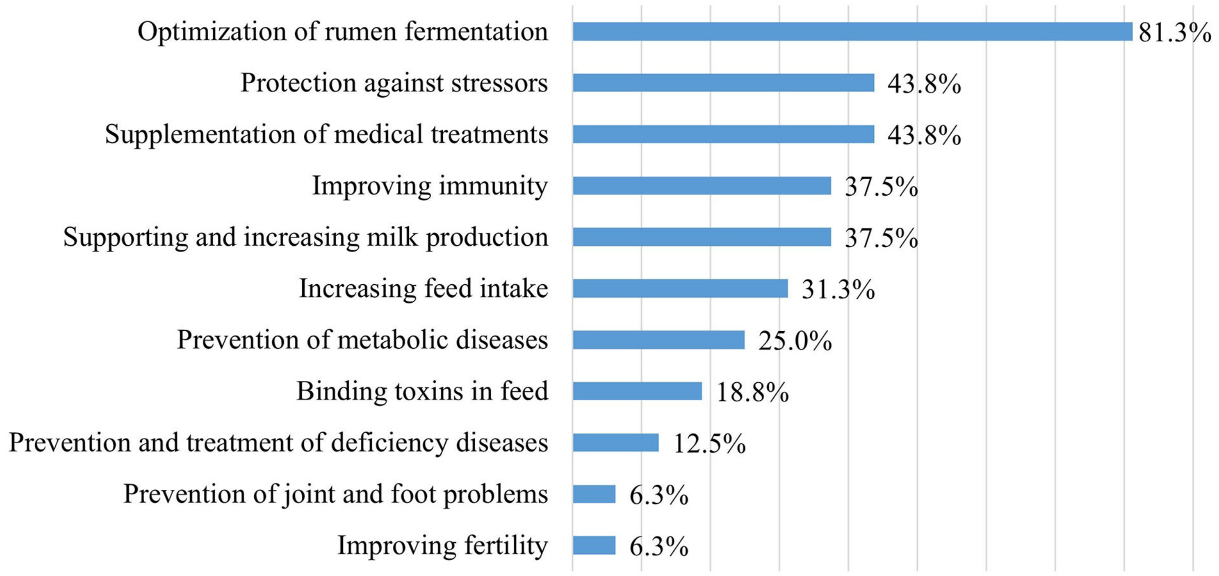

FIGURE 3
The purpose of using probiotics ($n = 16$).

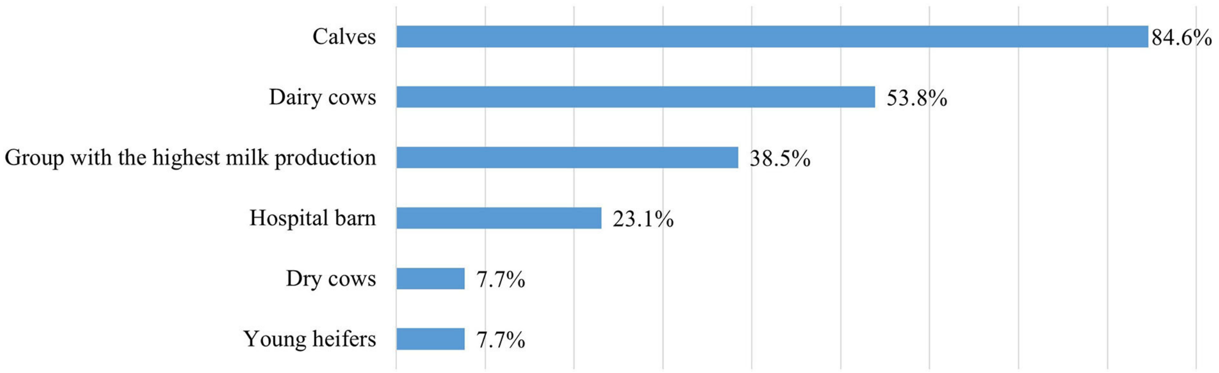

FIGURE 4
The use of probiotics by animal groups ($n = 13$).

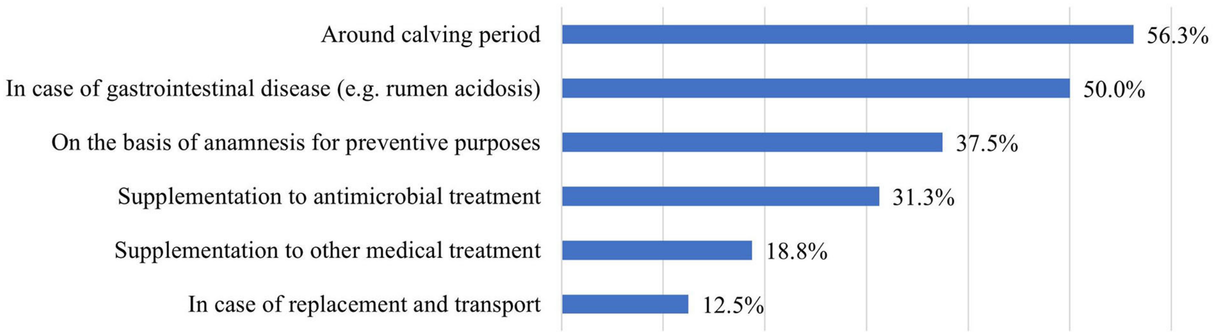

FIGURE 5
Indications for probiotic feed supplementation ($n = 16$).

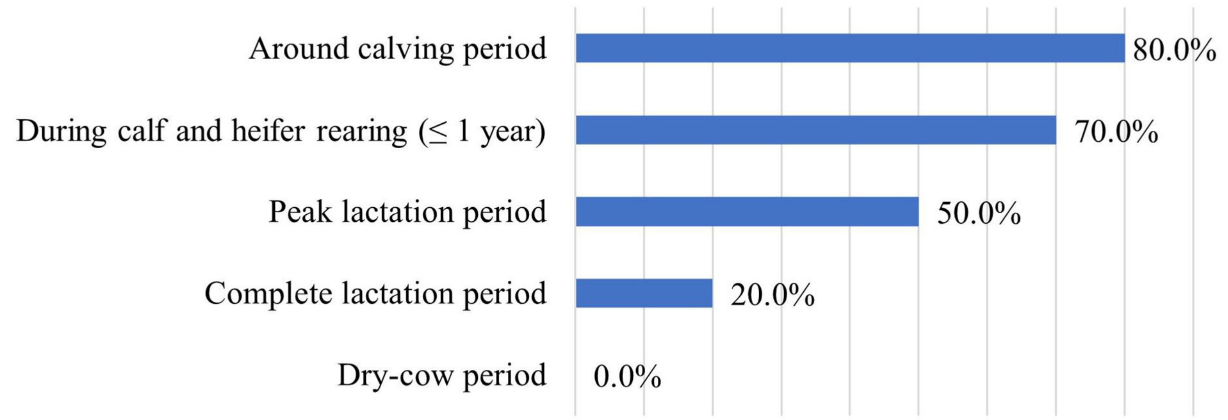

FIGURE 6
Indications for the periodic usage of probiotics (n = 10).

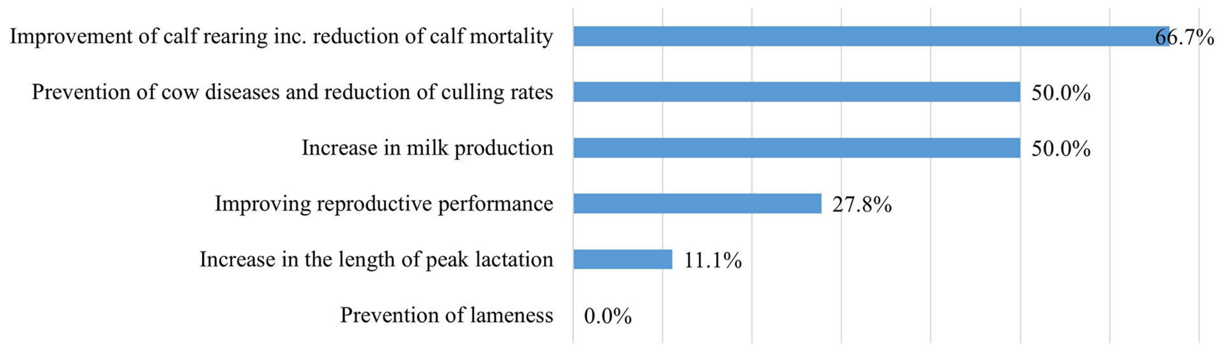

FIGURE 7
Expected effects of the use of probiotics (n = 18).

knew only 1–2 products and another one (4.3%) none. Most farm experts get to know the new probiotic products through sales representatives or from journal publications (Figure 9).

To the open-ended question, "*What do you expect from the new generation of probiotic products?*," several respondents (n = 7) would replace the current probiotic products with more effective, wider spectrum, easy-to-use, and better value-price formulations.

The production and distribution of probiotics

The surveyed feed distribution companies were founded between 1981 and 2010, and six out of eight (75%) were Hungarian majority owned and two (25%) were international majority owned. In 2017, two companies (25%) had net revenues between 323 and 1,617 thousand EUR (1 EUR = 309.21 HUF), two (25%) had between 1,617 and 3,243 thousand EUR, two (25%) had between 3,243 and 16,170 thousand EUR, and two companies (25%) had revenues over 32,340 thousand EUR. Considering the animal feed market share, two feed distributor companies were in the top 3 in Hungary, one was in the top 4–10 companies, while the other five were not among the top 10 companies in terms of turnover. Four out of eight firms (50%) exported feed to Asia, America, and Europe, primarily to Romania, Moldova, Austria, Slovakia, Russia, Georgia, and Iran. Six out of eight feed distribution companies also had feed production capacities, and all six companies produced feed for both cattle and pigs, five for poultry, four for rabbits, and three for sheep and goats. Two out of eight companies (25%) distributed feed additives only, not ready-made feed. The surveyed companies, that produce compound feed, produced on average between 3,000 and 800,000 tons of complete compound feed per year, of which between 60 and 25,000 tons were produced for cattle. Their main feed supplements for cattle included mycotoxin binders, protected proteins, rumen buffers, polysaccharide enzymes, and yeasts. In Hungary, the

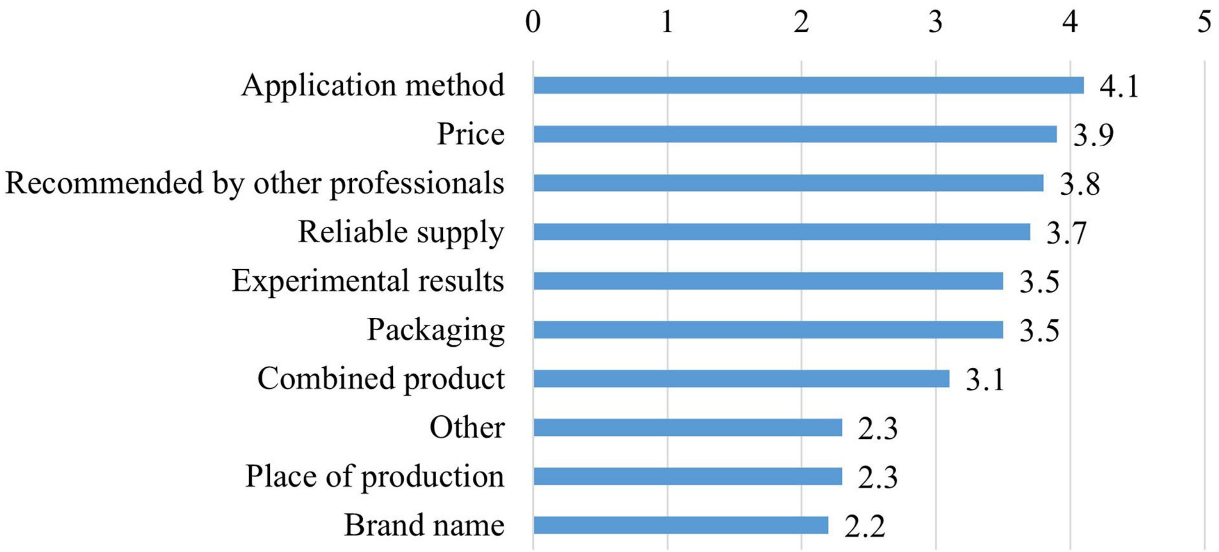

FIGURE 8
Importance of procurement factors for probiotics ($n = 19$). On a 5-point Likert scale, where 1 = not important at all and 5 = very important; combined products contain more than one probiotic component.

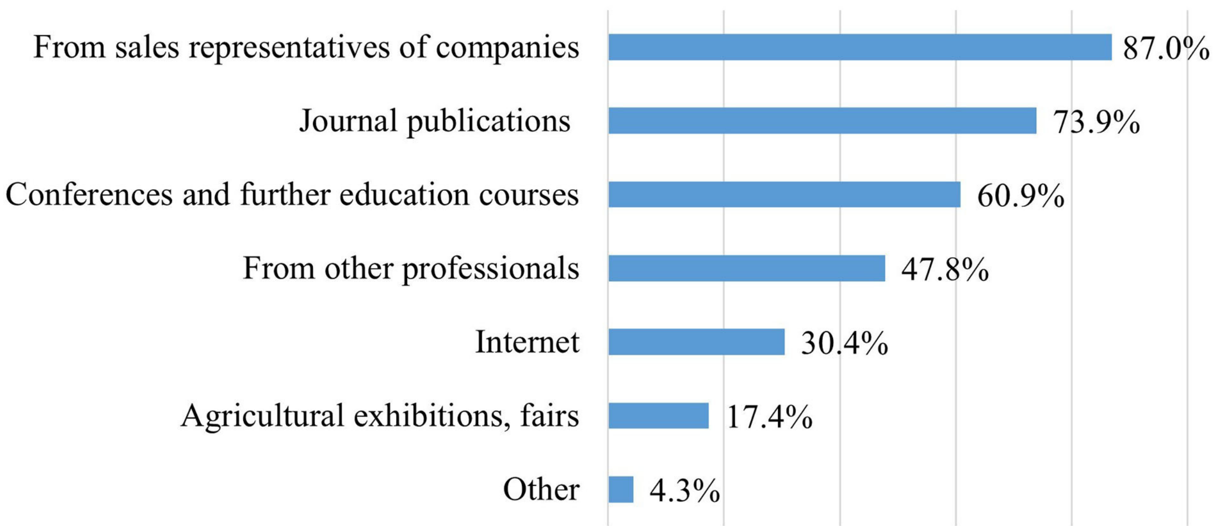

FIGURE 9
Sources of knowledge of new probiotic products ($n = 23$).

total complete compound feed production for food-producing animals was 3.526 million tons in 2017, out of which 350.5 thousand tons were produced for cattle (15).

All the eight surveyed feed companies distributed probiotic-containing products, and they started selling these products between 1988 and 2012. The six surveyed companies with feed production capacities produced between 5 and 130,000 tons of probiotic-containing preparations per year. Five out of these six companies produced probiotic products for cattle, on average 3,112 tons per year, and out of this amount, they produced on average, 3,091 tons per year for dairy cattle. Probiotic products for cattle contained specific live yeast or bacteria (e.g., *Enterococcus faecium*). The income from probiotic products was 27% on average of the total earnings from all feed supplements ($n = 8$; min 5%; max 80%). The income from probiotics for cattle accounted for 33%, on average, of the total income from all feed supplements for cattle ($n = 7$; min 0%; max 72%).

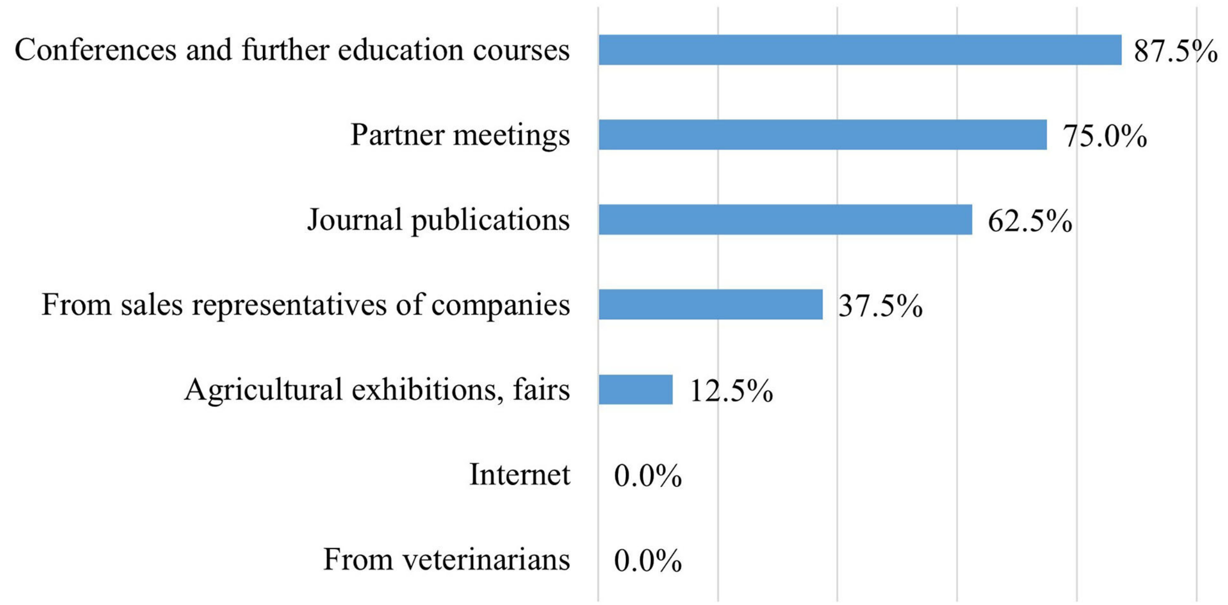

FIGURE 10
The methods considered most effective by feed distributors to increase the farm nutrition experts' knowledge of probiotics ($n = 8$).

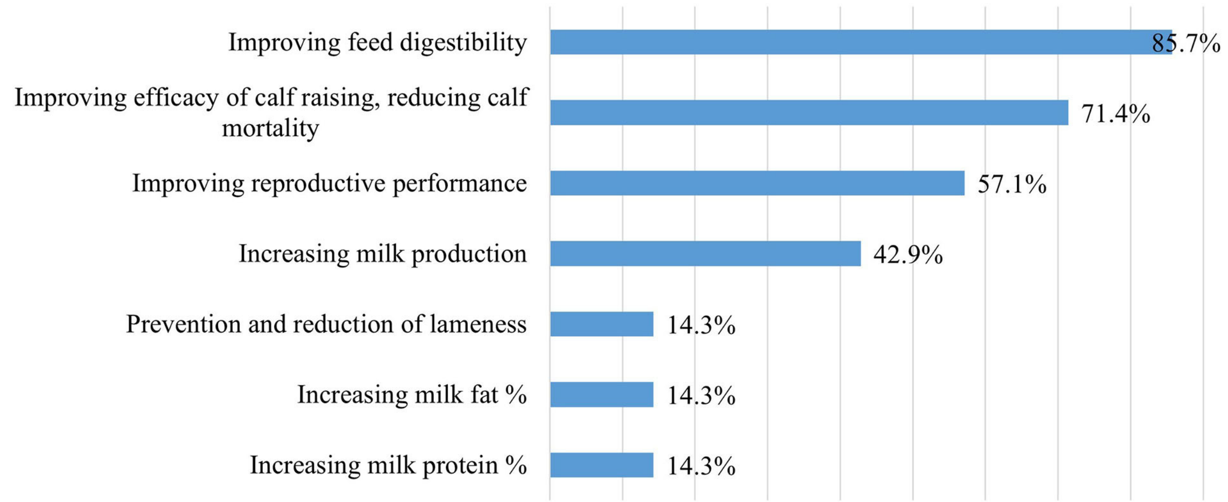

FIGURE 11
Expected impact of probiotic products in dairy cattle farms based on feed distributor companies' own experiments ($n = 7$).

Products available in Hungary during the time of this survey included feed supplements for calves, heifers, and adult cattle as well (mostly targeting peak lactation and heat stress periods). There were different application methods available (predominantly by drenching or mixing into milk replacer), based on the age of the animal, the type of treatment (individual or group level), and other components of the same product. Probiotic components included different bacterial strains (e.g., *Bacillus licheniformis, Bacillus subtilis, Enterococcus faecium*) and/or live yeast (e.g., *Saccharomyces cerevisiae*). Most common additives to probiotics included but were not limited to fructooligosaccharides, vitamins (A, D, E vitamins, biotin), minerals (e.g., manganese, selenium, copper, zinc), L-carnitine, rumen buffers, and enzymes. Some probiotics might contain GMOs. Some distributor companies provided detailed online product descriptions on their websites or in company journals, while others preferred to only provide a list of products or just a general summary of their professional activity and relied more on sales representatives to spread knowledge.

The managers from feed distributor companies were asked to evaluate the farm nutrition experts' awareness of probiotics. Two out of eight company managers (25%) perceived the knowledge of probiotics among the experts responsible for the farm feeding program as good, two (25%) rated it as average, three (37.5%) evaluated it as below average, one could not judge it (12.5%), and none of them perceived it excellent. This corresponds to the fact that more than half of the farm managers are of the opinion that they did not have sufficient information about probiotic products and their applications. According to the feed company managers, the most effective way to increase the farm nutrition experts' knowledge of probiotics could be through conferences, further education courses, or partner meetings (Figure 10). However, most farm experts learn about new probiotic products from sales representatives and journal publications.

Based on the own experiments of feed distributor companies, probiotic products were primarily expected to improve feed digestibility, reduce calf mortality, and leverage the cows' reproductive performance (Figure 11). This corresponds to the expected impact of probiotics, as per the opinion of farm nutrition experts, since many of them mentioned the prevention of cow diseases and increased milk production in addition to improving calf rearing efficiency.

According to the experience of feed distributor managers, the most important customer requirements for probiotic products were obvious performance improvements ($n = 2$) and good return on investment ($n = 2$), easy integration into technology ($n = 1$), pathogen control ($n = 1$), reduction of antimicrobial use ($n = 1$), improved feed conversion efficiency ($n = 1$), and reduced rumen acidosis ($n = 1$). After the use of probiotics, the feedback from customers showed a reduction in gastrointestinal diseases (e.g., rumen acidosis, diarrhea; $n = 3$), improved digestion of fiber ($n = 2$), increased milk production ($n = 1$), and less calf mortality ($n = 1$). No negative criticisms were received as regards to probiotic products, but it is important to note that where economic indicators cannot be properly evaluated, financial returns cannot be demonstrated easily, furthermore, the failures of herd health management cannot be avoided with these products.

In the feed distributor managers' opinion, the consumers had different expectations for the next generation of probiotics. These included the development of symbiotic (prebiotic and probiotic) products ($n = 1$), species specificity ($n = 2$), isolation from the digestive tract ($n = 1$), colonization at different parts of the gastrointestinal tract ($n = 1$), ease of use ($n = 1$), improved stability in feed ($n = 1$), and helping to reduce antibiotic usage ($n = 1$). According to the forecast of feed production companies, the next generation of probiotics will contain multiple strains and species of bacteria ($n = 1$), will not contain bacteria carrying antibiotic-resistant genes ($n = 1$), will be microencapsulated ($n = 1$), and will contain both pre- and probiotics ($n = 1$). The next generation of probiotics will reproduce faster in specific areas of the digestive system ($n = 2$) and will bind to the mucous membrane, i.e., they will also have an immune-stimulating effect ($n = 3$) and inhibit inflammatory processes ($n = 1$). Species-specific probiotics with competitive exclusion might be preferred ($n = 1$). Four out of eight companies (50%) did not develop probiotics and two of them (25%) had no local scientific partners, while one company (25%) could rely on the professional support of 1–2 Hungarian research institutes or universities, another one (25%) could have 3–5 domestic scientific partners in the development of probiotics.

The survey respondents' opinion was quite divided on the question of "what the proportion of dairy cattle farms in Hungary was, which did not use probiotic products yet." One company manager said that around 20% and selecting and evaluating the relevant product was a problem, as opposed to another one who put the figure at around 90%. This share depended on the target group of the products, for example, it was higher for calf nutrition, which is consistent with the fact that both herd managers and companies experienced and expected an improvement in the efficiency of calf raising in relation to the use of probiotics. In their view, in dairy farms that did not use probiotics yet, the application of probiotics could be encouraged by on-farm experiments ($n = 2$) and the distribution of probiotics which could be easily integrated into the farm technology ($n = 1$). In dairy farms, where probiotic products were already used, company managers did not think that the use of probiotics could be increased significantly ($n = 2$), but there would be a demand for better quality probiotic products and their proper application ($n = 1$). They were also convinced that the use of probiotics could be further increased in dairy farms as a part of preventive herd health programs ($n = 5$) and as additional treatments to the unavoidable, curative antibiotic medications ($n = 7$). Other indications for probiotic use might be dysbiosis ($n = 1$), digestive problems (e.g., rumen acidosis; $n = 3$), and lameness ($n = 1$). According to 87% of company managers, efforts to reduce antibiotic use could increase probiotic use. Accordingly, the surveyed feed distribution companies expected average annual growth of 54% in sales of probiotic products in Hungary over a 3-year-long period ($n = 7$; min. 4.5%; max. 300%).

Discussion

Based on the results of the survey, it can be stated that in the Hungarian dairy cattle farms, probiotic feed supplements were mainly used during calf raising and around the calving period, mostly to increase the efficiency of calf rearing, reduce calf mortality, and optimize rumen fermentation. However, the expected positive effects depend on several factors, such as the microorganisms used as the basis for the probiotic product, the animal-breeding, hygiene conditions on the farm, and the general health status of the animals (13). For instance, under relatively stress-free and temperature-controlled housing conditions, there was no significant difference in the body weight gain and the immunoglobulin levels of Holstein-Friesian calves, which were given milk replacer and starter diet, supplemented with a probiotic product containing *Bacillus* species, compared to the calves in the control group (16).

One of the most common indications of the use of probiotics was to optimize rumen fermentation. An analysis showed that in ruminants, yeast probiotics containing at least one strain of *Saccharomyces cerevisiae* significantly increased rumen short-chain fatty acid concentrations and rumen pH, but the results varied widely. The higher the proportion of neutral detergent fiber in the diet, the better the digestibility of organic matter (17). Yeast supplementation can increase rumen pH and volatile fatty acid concentration while reducing rumen lactic acid concentration (18). Furthermore, several studies reported that yeast-based probiotics in ruminants increased the number of cellulolytic bacteria, which resulted in higher cellulose degradation and microbial protein production (19–21). Pinloche et al. studied the effects of probiotic yeast supplementation in dairy cows at recommended and lower feed intake rates in the early period of lactation (12). Yeast supplementation resulted in higher rumen pH, significantly lower ammonia and lactate concentrations, and significantly higher concentrations of volatile fatty acids, propionate, and butyrate. These values were measurable at both moderate and higher yeast concentrations, but higher yeast concentrations led to better results (12). Probiotics products containing *Bacillus licheniformis* resulted in higher total rumen microorganism content, saturated fatty acid, and propionate concentrations, while the rumen had lower ammonia and lactic acid concentrations (22). According to several studies, probiotics have also been shown to be effective in the prevention or treatment of rumen acidosis. The yeast *Saccharomyces cerevisiae* reduced lactic acid concentrations in the rumen of dairy cows (23), which is likely to have inhibited the development of rumen acidosis (24). In contrast, Hristov et al. reported that *Saccharomyces cerevisiae* had no effect on rumen fermentation. Improvement of feed digestibility in ruminants can also be achieved by using probiotics (25). The use of yeast probiotics increases both fiber digestibility and protein turnover by increasing the number of cellulolytic bacteria in the rumen (19, 26).

Similar to the findings of our survey, several studies showed that certain microorganisms increase milk yield in dairy cows (27–29). Xu et al. investigated the effects of probiotics *Lactobacillus casei Zhang* and *Lactobacillus plantarum P-8* on milk production and milk composition. The use of these probiotic mixtures increased milk yield while reduced somatic cell count by positively affecting the composition of the rumen microbiota (30). Milk yield increased by 2.3 L per cow after daily supplementation with *Enterococcus faecium* (31). Feed supplementation with a combination of *Lactobacillus acidophilus NP51* and *Propionibacterium freudenreichii NP24* resulted in a 7.6% increase in average daily milk yield for Holstein cows (32). Poppy et al. and Maamouri et al. concluded that probiotics containing *Saccharomyces cerevisiae* increased milk production. Using yeasts as feed supplements can increase ruminant dry matter intake and milk production and can improve milk quality (33, 34). Lactic acid-producing bacteria significantly increase milk production, milk protein percentage, and non-fat dry matter content of milk, as well as reduce somatic cell count and mastitis severity by stimulating the immune system (18). Probiotic products containing *Bacillus licheniformis* significantly increased both the milk yield and milk protein content (22).

Several studies examined the effects of probiotics on calf growth and health, showing that probiotics improve average daily gain and feed conversion efficiency in calves (35–39). The fact that calves' probiotic supplementation was more widespread than that of cows is in line with the fact that a large proportion of research studies specifically examined the effect of probiotic supplementation on the body weight gain of calves (35). The feed intake and live weight of calves that were fed a starter diet containing *Saccharomyces cerevisiae* yeast culture were larger on days 42 and 56 of life compared to the calves in the control group and even to those that were given *Bacillus* species supplementation (40). Calves raised on probiotic-supplemented milk could be weaned earlier and had higher body weight at the time of weaning (41). Probiotics containing *Saccharomyces cerevisiae* were shown to improve growth rates in dairy heifers (42). Similarly, a strain of bacteria, *Propionibacterium jensenii 702*, increased weight gain in Holstein-Friesian calves by 25% in the pre-weaning period, and 50% in the weaning period (43). Frizzo et al. concluded that the use of lactic acid probiotic bacteria (e.g., *Lactobacillus acidophilus, Lactobacillus plantarum, Enterococcus faecium, Bifidobacterium* species) increased body weight gain and improved feed conversion efficiency in young calves (35). Probiotics increased the rate of weight gain in 1-week-old beef calves during the first 2 weeks of administration. The rate of increase in weight gain during the first 8 weeks was greater in calves that were less expected to be healthy. Probiotic treatment reduced the incidence of diarrhea, which reduced the need for antibiotic treatment and reduced mortality (44). In a survey by Kelsey and Colpoys, weaned calves were fed a probiotic-supplemented diet for 3 weeks. During this time, an improvement in average daily gain was reported in these calves compared to those not treated with probiotics (45). The improvement was attributed to the feed digestibility enhancing benefits of probiotics, which prevent excessive lactate production and normalize rumen fermentation (46). A study on 6-day-old dairy calves showed that *Enterococcus faecium M74* had a positive effect with significant improvements in body weight and daily weight gain over the entire study period of probiotic treatment (62 days). Probiotic treatment also reduced the incidence of diarrhea (39).

Overall, the most common indications of the use of probiotic products in the surveyed farms (e.g., optimizing rumen fermentation, protection against stressors, strengthening immunity) were mostly the same as those described by Chaucheyras-Durand and Durand (5), who highlighted that the most significant beneficial effects could be achieved during periods of stress for the animals and their intestinal flora (e.g., weaning), and the quantity and quality of milk production could also be significantly improved by probiotic supplementation of ruminant feeding. Certain *Lactobacillus* strains, in addition to their role in maintaining the balance of the intestinal flora, also have anti-inflammatory effects, and thus significantly decrease IL-6, IL-8, IL-10, and TNF-α production in the presence of LPS by reducing gene expression (47). Probiotics can also prevent rumen acidosis and relieve its symptoms by stabilizing rumen pH at a higher equilibrium value by reducing ammonia and lactate concentrations and increasing the concentration of volatile fatty acid, propionate, and butyrate (12, 22, 48). Thus, the main objectives of the use of probiotics in dairy cows are to increase milk production and to improve milk quality, feed conversion efficiency, and animal health status (e.g., reducing rumen acidosis), while in beef cows, the major objectives are to improve live weight gain, feed conversion efficiency, animal health status, and reduce pathogen excretion (5, 49).

Despite the seemingly positive production impact of probiotics, most of the surveyed dairy farms used probiotic preparations only intermittently and there was a significant number of farms that did not yet use probiotics at all, which represents a niche market for feed supplement distributor companies. In addition, while group-based use was more prevalent, individual feed supplementation may become more prevalent as precision livestock farming gains ground. In addition to increasing the quantity of probiotics used, emphasis should also be placed on the proper use of probiotic products. As the questionnaire responses showed, although the farm nutrition experts being responsible for the feeding programs were aware of several different probiotic products, they were often not sufficiently informed on how to use them properly.

The role of probiotics in the fight against antibiotic resistance could also be very useful; however, they might have potential adverse effects. Shridhar et al. (50) used a whole genome sequence-based analysis to detect antimicrobial resistance genes and their results showed that *Enterococcus faecium* carries genes that confer resistance to antibiotics, which are widely used in human medicine (including aminoglycosides, macrolides, lincosamides, tetracyclines, and phenicols). Thus, by treating animals with probiotics, the genes could be transferred to pathogenic bacteria and make them resistant to antibiotics that can be passed on to humans. In the future, probiotic preparations may need to be tested for antimicrobial resistance genes before they can be marketed to food animals (50). In addition, *Bacillus cereus* also produces enterotoxins and emetic toxins (9). Probiotics might also be responsible for systemic infections, adverse metabolic activities, excessive immune stimulation, and gene transfer in the host due to the production of harmful substances by probiotic microorganisms (51). Therefore, there is an urgent need to molecularly investigate the long-term (5–10 years) effects of probiotic microorganisms on the gastrointestinal mucosa (6).

Our results showed that for the probiotic producers and distributors in Hungary, the goal in the product development of probiotic feed supplements is to create more effective, easy to use on herd level probiotics with a wider indication spectrum and better value-price ratio, than the available products in the market. Thus, there was a need for more complex feed supplements, which, for instance, contain both prebiotics and probiotics. The supplementation of feed with fermented wheat germ extract as a prebiotic for suckling dairy calves resulted in a significant reduction in the incidence of respiratory, gastrointestinal, and other diseases, as well as in the use of antimicrobials, and caused an improvement in body weight gain (52). The fermented wheat germ extract supplementation for beef calves brought about a significantly higher live weight gain and lower morbidity and calf mortality rate, and finally, a reduction in the use of antibiotics (52). However, Heinrichs et al. (53) found no significant improvement in calf health (diarrhea, respiratory diseases, general health status) in the prebiotic-supplemented group of calves, but the control group had two times as many calves with diarrhea, and their feed intake was also significantly reduced. The intestinal flora did not differ largely, but the calves in the control group had slightly more *Enterobacter* species, while those in the prebiotic-supplemented group had more *Lactobacillus* species.

According to the questioned farm nutritional experts and probiotic distributor managers, as the use of antibiotics is restricted and must be reduced, probiotics could be brought to the fore as part of the preventive herd health programs instead of the widely used antimicrobial metaphylactic treatments and could more often be complements to the necessary, curative antibiotic treatments. However, the gut microbiota is complex, and it is not yet fully understood how the effects of bacteria benefit the host. Active research is ongoing on the effects of probiotics on live bacteria. Probiotic bacteria have a positive effect on digestive tract function in ruminants by benefiting the microflora and suppressing known gut and food-borne pathogens. But their efficacy and mechanism of action need further investigation (54). Based on the respondents' opinion, the most effective ways to share the newest knowledge about probiotics with farm nutritional experts are the different personal meetings.

According to our knowledge, this was the first scientific study assessing the use of probiotics in nutrition and herd health management in large Hungarian dairy cattle farms, but the limitation of the survey is the non-representative nature of the sample.

Data availability statement

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

Ethics statement

The revised survey was reviewed by the Scientific Research Committee of the Faculty of Veterinary Science, Budapest and found exempt from human subject protection regulations. The participants provided their written informed consent to participate in this survey.

Author contributions

LÓ and ZV conceived and designed the study. ZV collected and analyzed the data. LÓ, MM, and ZV contributed to the conceptualization and writing the article. LÓ acquired funding. All authors contributed to manuscript revision, read, and approved the submitted version.

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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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2022.957935/full#supplementary-material

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Bovine tuberculosis control in Fiji: Retrospective study findings for 2015 to 2020

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Control of bovine tuberculosis (bTB) is a priority for animal health, biosecurity, and human health authorities in Fiji as evident from the long-term funding of the Bovine Brucellosis and Tuberculosis Eradication and Control program (BTEC) and notable improvements to the program described in this paper. To evaluate the performance of the Fiji BTEC program from 2015 to 2020, all available bTB data for cattle were analyzed. Data sources included BTEC bTB testing records, abattoir records and laboratory records. We integrated all information to quantify the bTB tests applied, bTB positive farms and animals, meat inspection and laboratory findings. Test coverage was highest among dairy cattle in Central Division (∼73%), where bTB was highly prevalent with 7.8% of dairy cattle and 61.7% of dairy farms found to be positive between 2015 and 2020. There was no visible downward trend in the apparent prevalence of bTB over the 6-year period. During 2019 and 2020, only 21.3% (51/239) of the tested dairy farms maintained their clear status, another 8.4% (20/239) reverted to infected status after 1 year or more of being bTB clear, and most farms remained infected during these 2 years. Factors observed to be contributing to this situation were persistent infections, related in part to the significant number of untested animals, uncontrolled animal movements, and larger farm size. Similar to other developing countries, bTB remains a serious concern and further strengthening of the program targeting the main contributors to bTB persistence, along with maintenance of a comprehensive reporting and traceability system, industry awareness and government support are needed. Control of bTB in Fiji is a long-term objective that must have multiple stakeholder engagement and regular review to measure success.

KEYWORDS

bovine tuberculosis, disease control, cattle, BTEC, Fiji

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Introduction

Bovine Tuberculosis (bTB) is primarily caused by *Mycobacterium bovis* and is a chronic disease that constitutes a significant economic burden to cattle production industries (1, 2). bTB is a geographically widespread infectious disease (https://www.cabi.org/isc/datasheet/91739). It is transmitted between cattle and people, and cattle and wildlife making control a substantial challenge for public health and animal health systems (3, 4). Programs to control bTB in cattle are generally based on test and cull, implementation of hygienic practices, certification of bTB-free farms and slaughter-based surveillance; the components vary according to the goals of the control program in a particular country and the level of disease therein (5). In high-income countries where control programs have been sustained for lengthy periods, progressive reduction in distribution and prevalence has often been achieved. However, the eradication of bTB from cattle populations (extinction of *M. bovis*) has been achieved by few countries, notably Australia (6). In terms of legal freedom, several countries and regions across Northern Europe, Asia, and America are declared free of bTB based on bTB testing over a period of 3 years or more with no evidence of bTB in at least 99.8% of farms representing 99.9% of the total cattle population (7–9). *M. bovis* in wildlife reservoirs is described to be the main reason for the persistence of the disease hampering biological freedom of bTB in high-income countries such as New Zealand, UK, and USA (6, 7, 10, 11). In most middle to low-income countries where bTB is endemic, even planning a disease control program can be a challenge due to insufficient data collection to determine the epidemiological situation, which is essential information to effectively allocate scarce resources for bTB control (12–14).

Globally there is a renewed focus on control of bTB because it is linked to the World Health Organization (WHO) strategy to end the human tuberculosis (TB) epidemic with targets for large reductions in TB incidence, TB-related deaths and household costs by 2030. People at risk of zoonotic TB are often members of neglected populations deserving greater attention. Thus, a Roadmap for Zoonotic TB was endorsed by WHO's Strategic and Technical Advisory Group for TB in 2016 (15).

In Fiji, the need to consider zoonotic TB in the national TB program is reflected in the designation of bTB as a One Health challenge by the Fiji Ministry of Agriculture (MOA) and the Ministry of Health and Medical Services (MHMS) (16). The recognition of the detrimental impacts for animal health and human health has motivated research, collaboration and information sharing by the two ministries.

In fact, control of bTB has been a priority for the Government of Fiji for more than two decades, evidenced by the consistent annual funding of the Bovine Brucellosis and Tuberculosis Eradication and Control program (BTEC). Cattle are highly valued in this Pacific-island nation, which despite limited farmland (10.7% of total aggregate land area) raises a total of 119,691 cattle (70,041 beef cattle and 49,650 dairy cattle) under extensive grazing management according to the 2020 Fiji Agricultural Census (2020 FAC) (17). This is one head of cattle for every eight people in Fiji based on the 2019 human population total of 906,784 (17, 18). The dairy industry is particularly significant for the country, providing 79.5% (1,696,695 liters of fresh milk) of total volume for sale according to a 3-month analysis by the 2020 FAC (17). Whilst the BTEC program involves conduct of tests for brucellosis and for tuberculosis during a farm visit, this study focused solely on bTB data, in part because there were no positive brucellosis test results during the 6-year study period. This study is one example of the work undertaken, and it addresses a priority area of the Roadmap for Zoonotic TB to improve the scientific evidence base: surveillance and reporting of better quality data on bTB in livestock (15). Zoonotic aspects of bTB in Fiji will be reported separately.

The key activities of the Fiji BTEC program over time have been mandatory on-farm bTB testing using the single intradermal test (SID) with unique individual identification of tested animals, data recording and analysis; removal of infected cattle from farms with compensation; laboratory and abattoir surveillance; and cattle movement control to reduce and prevent infection spread from infected farms. However, a retrospective investigation of BTEC bTB data drawn from hard-copy records for the 16 years from 1999 to 2014 demonstrated that despite sustained funding of the BTEC program, disease reduction and containment was not being achieved (19). This finding provided the needed justification for the MOA to implement changes in 2014 in the reading of the SID to improve the sensitivity of detection of infected animals in the field. In 2016, the Biosecurity Authority of Fiji also implemented movement restrictions through the Biosecurity Emergency Area Declarations that states that any movement of cattle and calves within Fiji is strictly prohibited unless BAF provides prior authorization for the movement. Farmers must complete the required cattle movement application form and submit it to a BAF office at least 7 days before the movement date. A farm's bTB status, which is determined by the MOA's BTEC team based on BTEC on-farm testing results, must be a "Clear Status" for BAF to issue the movement permit.

These changes had some economic consequences for the dairy industry, such as a reduction of herd size on some dairy farms due to culling of bTB positive animals (also called reactors) and led to concern about the limited replacement stock being available in Fiji. Furthermore, as the prevalence of animals with generalized and gross bTB lesions in infected farms decreased, a greater proportion of culled reactors had no-visible lesions (NVL) on post-mortem inspection at the abattoir. All this raised questions about the sustainability of using the SID testing protocol (20). As a response to farmer concerns, the first BTEC stakeholder's forum was organized by the MOA in 2017 to encourage participation of the industry in making decisions for implementation of the BTEC program. One of the important outcomes of the 2017 forum was the endorsement of a new BTEC Strategy by industry stakeholders in 2018. This strategy included enhanced criteria for the identification of infected cattle farms based on the SID test, immediate removal of infected cattle with an improved compensation scheme, upskilling of meat inspectors, and strengthened implementation of restrictions on cattle movements.

Under the new strategy, BTEC information was intentionally incorporated into the design of a new cloud data platform *Bovibase*, to record farm details on location, production, livestock, farm testing and cattle movement, with the intention of data sharing using *Bovibase* as a national recording system for the cattle industry. *Bovibase* was launched in 2019 during a 2^{nd} BTEC forum, enabling the recording of on-farm testing, infectious status, and meat inspection results for bTB reactor animals. Functionally, BTEC staff were able to access information about farms that were due for bTB testing, thereby aligning testing dates and reading dates, and prioritizing infected farms. Furthermore, data for animals with an incomplete bTB test and missing animals were recorded.

This paper presents a detailed collation and analysis of BTEC bTB records from 2015–2020, providing estimates of the coverage of the BTEC program and of the level of bTB infection in cattle herds in Fiji. Furthermore, it provides evidence of factors that are contributing to the transmission and maintenance of bTB in dairy cattle and guidance for updating the BTEC strategy based on the progress that has been made to date. These findings extend beyond the previous retrospective study for 1999–2014 and demonstrate the need for continued investment and collaboration between the government and the cattle industry in Fiji to achieve sustained control of bTB.

Materials and methods

A retrospective study was conducted using bTB test data of the Fiji BTEC program from 2015 to 2020. Approval from the Ministry of Agriculture (MOA) to conduct data analysis was received in March 2020. Data preparation and analysis were conducted from November 2020 to October 2021. The diagnostic test used throughout the 6-year period was the SID test using purified protein derivative tuberculin antigen for *M. bovis* (PPD-B) injected intradermally into the caudal skin fold of the tail (CFT), with the result read 3 days after administration. Over the 6-years, it was deemed to be a positive test if the animal had developed a wheal of any size or redness at the injection site, following the OIE recommendation for detection of reactors in known infected farms (2). This test reading criteria, based on the assumption that all cattle in Fiji are potentially bTB infected, was implemented from 2014 regardless of whether a farm was previously identified as disease-clear or infected status (21).

The determination of clear or infected status was based on the following criteria: farms with reactors determined by the SID were classified as "Infected"; and for an infected farm to be determined as "Clear" from bTB, it was necessary for it to have 3 consecutive negative SID tests at a minimum of 3-month intervals to obtain "Restricted', "Provisionally clear," and then "Clear" statuses, respectively. Upgrading to a Clear status also relied on the compliance of the farmer to present all cattle older than 3 months of age for complete bTB testing (i.e., presented for tuberculin injection and then again for reading of the result) (21).

Data sources, bTB test data entry and verification

Several types of data were collated and used in this study (Figure 1). Due to weak record management systems and the collation of data from multiple sources, the processes for data collation, cleaning and verification were complex and time consuming, with this work extending over 11 months.

Soft copies of BTEC bTB test data from 2015 to 2017 encoded in Microsoft Excel were provided by the MOA. For 2018, a soft copy of some data was provided, and the remainder was subsequently encoded in MS Excel by BTEC personnel. The data from 2015 to 2018 were systematically checked using MS Excel (Microsoft® Excel® for Microsoft 365 MSO (16.0.14326.20908)) and R (version 4.0.4) to remove duplicate records and correct or delete errors after comparison against hard copies. Verification was performed by cross-checking against the unique identity farm (see below).

For 2018, a marked difference in totals was identified between verified bTB testing data, the BTEC annual report and the abattoir reports of bTB positive animals detected on farm and subsequently sent to slaughter. Consequently, the total time period for data verification was lengthy (from January to September 2021) and included an additional search of BTEC hard copy records and of soft copy files to exhaust all possible data sources, but no other files were found. Therefore, it was assumed that some 2018 test data records were permanently missing.

For 2019 and 2020, complete data were downloaded from the BTEC program section of the cloud data platform *Bovibase*. The data provided by *Bovibase* were verified by cross-checking against the hard copies and confirmed to be correct.

For farms visited only during 2015 to 2018, farm identification was based on unique farm names linked to the MOA dairy registration number or village, settlement,

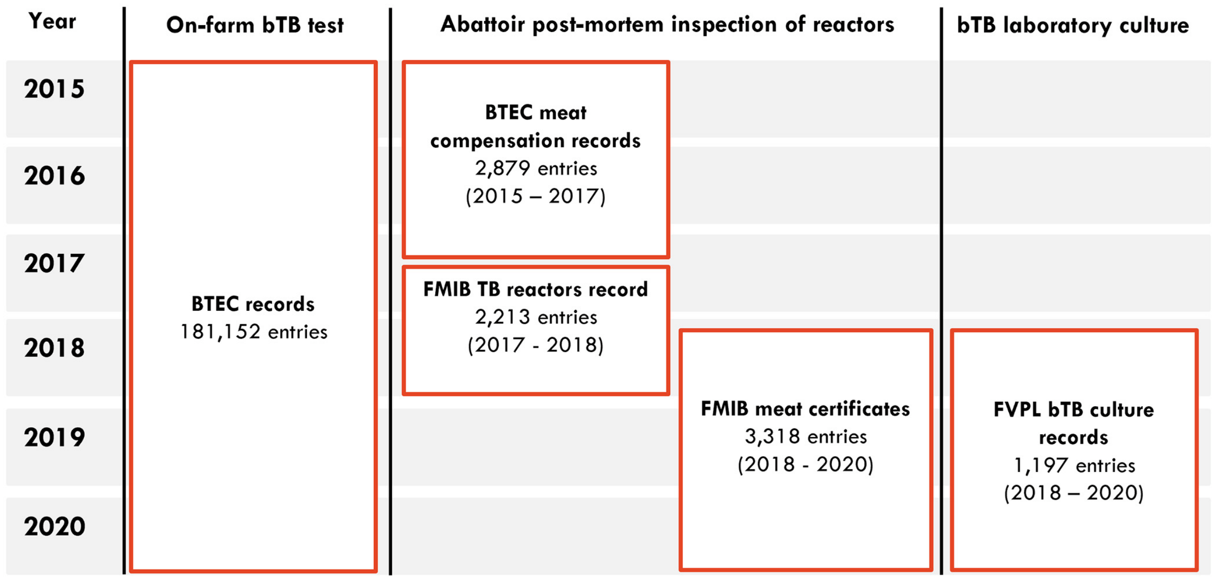

FIGURE 1
Fiji BTEC data sources used in this study from 2015–2020.

or town supply descriptions. For farms visited during 2019–2020, those data consistently contained a unique farm identification code (*Bovibase* ID) for each farm visited, and for farms that had also been visited previously during 2015 to 2018, the *Bovibase* ID was added to the earlier records.

Dairy farm identification was verified by matching the BTEC farm registration number and the farm name to the MOA dairy farm registration list; as each farm was required to register with the MOA on an annual basis and a new, different registration number was allocated each year, this prevented the analysis of data for a specific farm across all the years of the study. Following introduction of *Bovibase*, each dairy farm visited by BTEC in 2019 received a new, unique *Bovibase* ID that was maintained from 2019 onwards.

Validation of beef farms was based on familiarity of BTEC staff with farmers and farm operations as the MOA had no formal registration system for beef farms. From 2019, a unique *Bovibase* ID was allocated to each beef farm.

A list provided by the MOA Economic Planning and Statistics Division (EP&S) was used to validate location details recorded for each farm.

In the results section, the term "Unique identity farm" was used to indicate the count of unique *Bovibase* ID or farm name. There were 1,280 unique identity farms (dairy, beef and holding facilities).

All the data compiled and used for further analysis are described in Supplementary Table 1.

National cattle population data

As a national program, BTEC is responsible for investigation of cattle in Central, Western, Northern and Eastern divisions of Fiji (Figure 2). BTEC program population coverage of testing was estimated using the national cattle population figures from the 2020 Fiji Agricultural Census (15) as the denominator.

Identification and classification of farms

Farms of individual farmers, school farms, villages/settlements, government stations, cattle traders and impoundments were classified by type of operation (dairy, beef, holding facility). Dairy refers to an establishment that produced milk for a bulk supplier, town supply or for personal consumption, and/or traded dairy cattle. Beef refers to an establishment that only raised and/or traded cattle for meat production. Holding facilities refers to a site designated for holding impounded straying cattle with and without ear tags for short periods of time, (commonly called "pounds" or "impoundments)".

Dairy farms that had registered and paid a license fee to the MOA to officially sell milk to Fiji Dairy Limited (FDL) are referred to as licensed dairy farms. These licensed farms must comply with additional regulations related to hygiene and sanitary standards to be authorized to sell milk. BTEC prioritizes the testing of these farms under the BTEC program with the

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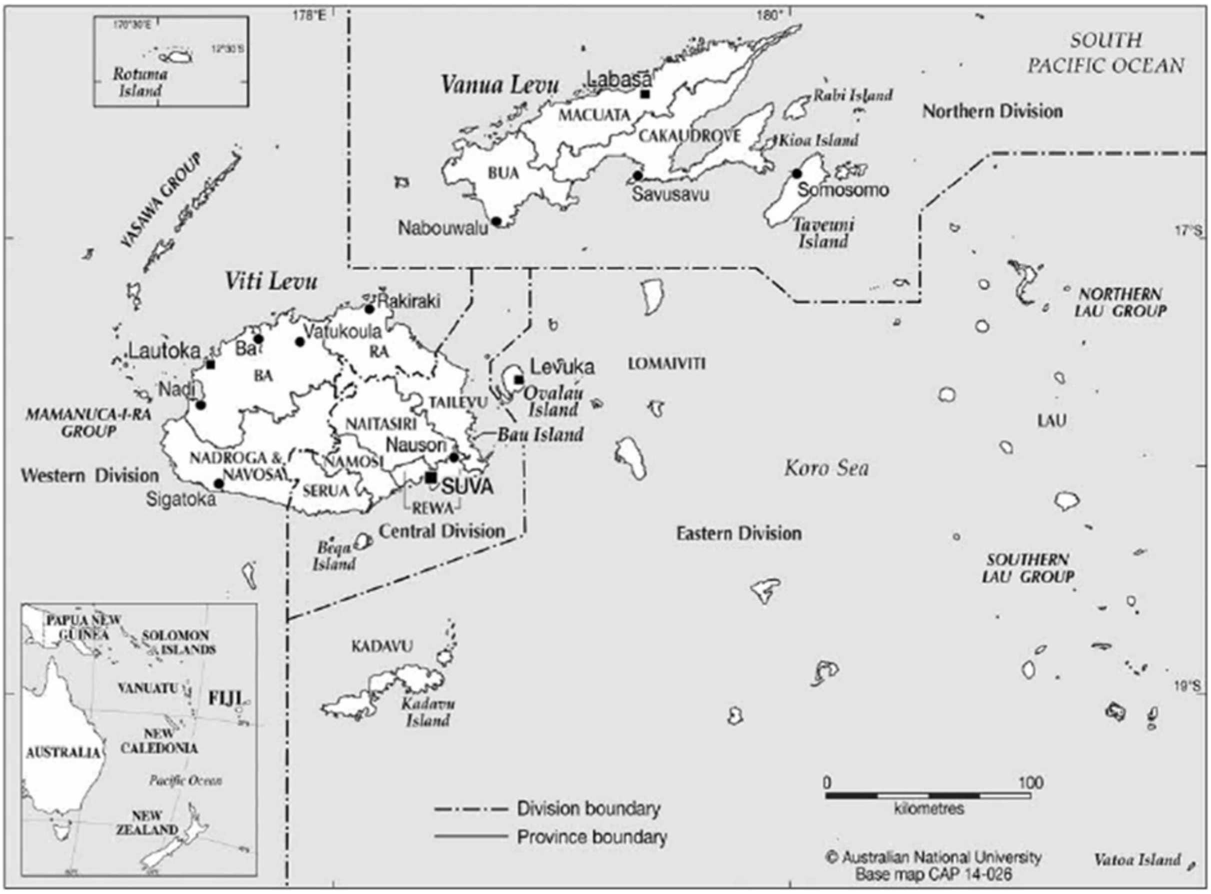

FIGURE 2
Map of Fiji showing Division boundaries. Maps Online, CartoGIS Services, ANU College of Asia and the Pacific, The Australian National University.

aim to improve national dairy production and the safety of their dairy products. Among these licensed farms, the commercial farms that usually have higher milk production are called bulk suppliers, and Fiji Dairy (PTE) Limited collects the milk from the farm using the company-owned bulk tankers to transport it to the milk processing factory at Nabua, Fiji. For the smaller licensed dairy farms, the collection of milk from the farm in aluminum milk cans is coordinated by the Fiji Cooperative Dairy Company Limited (FCDCL) which operates chilling centers where the milk is stored for cooperative members until collection by Fiji Dairy Limited.

Farms were classified based on herd size as commercial, semi-commercial or subsistence. A commercial farm was a farm with more than 40 cattle, a semi-commercial farm was a farm with 16 to 40 cattle and a subsistence farm was a farm with 1 to 15 cattle. Herd size was based on the median number of cattle bTB tested across the visits to a farm. If a farm was tested during 2015 to 2018 only, the median was calculated based on the total cattle tested per test visit during that period. If a farm was tested during 2019 to 2020, when untested animals were not counted, then the median was calculated based on the total cattle tested per test visit over these 2 years.

Individual animal data

Individual animals were identified by metal ear tags each with a unique number provided by BTEC. In the event that a tag was lost a new tag was provided by the BTEC Program. In the results section, the term "Unique identity animal" was used to indicate the unique metal ear tag animal identification listed on BTEC records.

On-farm testing

From 2015 to 2017, bTB SID tests were performed on animals older than 6 months and from 2018 onwards the tests were performed on animals older than 3 months as part of the enhancement of the standard operating procedures (SOP). For 2015 to 2018, it was assumed that all bTB SID test administered on Day 1 were read 3 days after the day of PPD-B tuberculin administration (Day 4). From 2019 the actual reading date was recorded in *Bovibase*, as well as the unique identity animal number of the animals that were not presented on the reading date (recorded as "Not Presented" or "NPD4"). The unique identity animal for animals that were presented by the farmers on Day 4 but that were not presented for testing on Day 1 were also recorded from 2019 (recorded as "Not Tested)". The term "Untested animal" was used to describe both "Not Presented" and "Not Tested" animals.

Furthermore, during 2019 and 2020, *Bovibase* was enhanced to classify unique identity animal numbers into a category called "missing animals" when not presented on Day 1 and Day 4 during a BTEC visit, without record of death, slaughter, or sale. When an animal was recorded as missing during 3 consecutive BTEC tests on a farm it was subsequently removed from the farm total stock.

The dataset compiled from 2015 to 2018 included: date of test, animal identification number (metal tag), animal type (milking cow, bull, bull calf, cow, dry cow, heifer, heifer calf, steer), animal gender (female or male), farm registration number, farm name, farm location (division, province, village/settlement, locality) and bTB SID results (Positive, Negative). From 2019 to 2020, the dataset also included: farm *Bovibase* ID, animals not presented on Day 4 for bTB test reading (NPD4), and animals not tested (Not Tested), date of reading, batch number and next test date.

Abattoir post-mortem inspection of reactors

Complete data for the post-mortem inspection of all reactors, issued by the meat inspectors of the MOA regulatory unit, were available only for the FMIB Nasinu abattoir. However, these records were not available for 2015 and 2017, thus Meat Compensation records issued by BTEC were used instead (Figure 1). These records included: unique identity animal number, unique identity farm, farm name, farm location, animal color, gender, stock movement number, type of lesion detected, payment rate ($), compensation percentage (%), total compensation ($) and lesion weight (kg).

From 2017 to 2020, records of the reactors' post-mortem inspection obtained from the FMIB Nasinu abattoir were, the "TB reactors record" from 2017 to 2018, and the "Meat Certificates" from 2018 to 2020 (Figure 1). TB reactors record and Meat certificates datasets included: animal identification number, farm registration number, farm name, farm location, animal type, animal gender, stock movement number, date of slaughter and type of lesion detected. Meat certificates provided additional data such as the meat certificate number and lesion weight for inspections done before 2019 and dressed carcass weight for inspections done from 2019.

There were two major categories of outcome for bTB reactors recorded by meat inspectors at the abattoir: no visible lesions of bTB (NVL) or visible lesions of bTB (i.e., gross pathological lesions) observed at post-mortem inspection. The type of bTB lesion was defined as: focal or generalized. Focal lesions were found in one area either in lungs, head, or lymph nodes such as prescapular lymph node, popliteal lymph node, internal iliac lymph node, mesenteric lymph node or superficial inguinal lymph node. Generalized lesions were recorded when there were very extensive bTB visible lesions in one region of the carcass or visible lesions in multiple locations. Visible lesions in the liver were also classified as generalized bTB due to the high risk of a bTB systemic infection.

Laboratory culture

Granulomatous lesions found in any cattle during post-mortem inspection at the FMIB Nasinu abattoir were sampled for submission to the Fiji Veterinary Pathology Lab (FVPL). If there were multiple masses, up to three masses were sampled. For bTB reactors with no visible bTB lesions, a minimum of three samples from lymph nodes were collected: for example, a sample of the lymph nodes from the head, lung and liver, or any other enlarged lymph node.

Records for sampled individual bTB reactors and non-reactors in 2018 and sampled bTB reactors from 2019 and 2020 were obtained from the FVPL. The laboratory records did not distinguish between gross lesions from a bTB reactor or a non-reactor, and it was not possible to verify against any other data source in this study. Since the laboratory was carrying out confirmatory tests for *M. bovis*, during 2019 and 2020, only samples from reactor animals with NVL were cultured to properly allocate the use of limited laboratory reagents and consumables, and samples from bTB reactor and non-reactor animals with gross lesions were not cultured. Samples of visible bTB lesions and NVLs were cultured in Löwenstein-Jensen pyruvate agar and Löwenstein-Jensen glycerol agar. Suspensions from culture plate colonies were prepared on glass slides, Ziehl-Neelsen staining was carried out and acid-fast bacilli were confirmed by light microscopy, enabling a presumptively culture positive classification. The FVPL dataset included: bTB culture line number, date of culture, animal identification number, laboratory case number, farm name, abattoir sample type (NVL, lung bTB, head bTB, popliteal bTB, generalized bTB, liver bTB), Löwenstein-Jensen pyruvate agar result, Löwenstein-Jensen glycerol agar result, Ziehl-Neelsen staining result and date read.

Animal movement

For 2019–2020, animal movements were identified based on a unique identity animal being present on different farms. The following types of movements were studied: animal movements from non-clear farms to a farm in a different province; animal movements from non-clear farms to a farm in the same province; animal movements from clear farms to a farm in a different province; and animal movements from clear farms to a farm in the same province.

Data analysis

All data analysis was performed using R (version 4.0.4).

Budget allocation for each year of the study was calculated as the sum of monthly estimates of the BTEC program operations that included the bovine tuberculosis and brucellosis testing performed during the same farm visit, from January to December.

The number of bTB tests conducted was the sum of the number of tests done each year.

BTEC program coverage for 2019 and 2020 was calculated from the number of unique identity animals with a complete bTB test as numerator and the Fiji Agricultural Census 2020 animal count as denominator. Coverage was tabulated by division and by type of farm operation.

Annual and 6-year figures on the proportions of infected unique identity farms and animals were calculated at national, divisional and provincial levels, and by type of farm operation. Unique identity farms were used to identify previously and newly enrolled farms in the BTEC program and to calculate their bTB apparent prevalence.

Further analysis of dairy farms was done including the detection of bTB by size of dairy farm operation, geographical regions, and the number of bTB test visits. The proportion of licensed dairy farms was investigated using Dairy Inspection department records.

The proportion of incomplete on-farm testing outcomes in 2019 and 2020 was calculated according to the number of farms with untested animals across all the farms tested by BTEC. The proportion of incomplete on-farm testing was tabulated by division and by dairy and beef farm operation types.

For farms tested in 2019 and 2020, the proportion of missing animals was calculated based on the median number of animals tested and missing per BTEC testing visit during each year.

From 2015 to 2017, the unique identity animal number from BTEC Meat Compensation records was used to count the number of reactor animals sent to the abattoir; all duplicated unique identity animal numbers were excluded (Figure 1). From 2018 to 2020, the unique identity animal number was used to count the number of reactor animals sent to the abattoir with the one following exception: in the event of a duplicated unique identity animal among the meat certificate records (Figure 1), a detailed check of the carcass weight, type of lesion detected, and meat certificate number was undertaken, and if all identical one of the duplicated records was deleted, but if different the separate records were retained.

The proportion of positive bTB cultures was calculated for reactors with NVL.

TABLE 1 Budget and number of tests performed by the BTEC program in Fiji.

Year	BTEC budget (USD)	Number of bTB tests
2015	482,279	18,986
2016	482,279	17,252
2017	482,279	28,309
2018	916,310	25,566
2019	1,350,340	37,020
2020	1,350,340	45,244
Total	5,063,827	172,377

During 2019 and 2020, unique identity animals that were present on more than one farm were used to calculate the proportion of animal movements from bTB clear and non-clear farms.

Results

The available data on bTB included in this study cover the period 2015 to 2020 and so includes periods during and after implementation of the new SOPs from 2014 and the enhancements to *Bovibase* from 2019.

Budget and bTB testing

The cumulative budget for BTEC program operations addressing bTB and brucellosis from 2015 to 2020 was 5.06 M USD (Table 1) with an average of 0.84 M USD budget per year. There were increases to the budget of 47.4% in 2018 and 32.1% in 2019. The increase in annual funding was associated with an increase in testing. For 2018, the apparent lower number of tests was due to missing bTB testing farm records.

National, divisional, and provincial coverage of Fiji's BTEC program 2015–2020

From 2015 to 2020, a total of 3995 farm visits were conducted by the BTEC program across the 4 Divisions of Fiji. This corresponded to 1,280 unique identity farms, the vast majority of which were tested more than once in this period. On average 172 new farms (median 209; range 78–236) were enrolled each year from 2016–2020. On average, 21,560 cattle (median 21,481; range 13,894–28,616) from 455 farms (median

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TABLE 2 Number of unique identity farms and animals tested each year for bovine tuberculosis by the Fiji BTEC program from 2015 to 2020.

Year	No. farms[a]	No. animals[a]	No. newly enrolled farms	Positive tests			
				Farms		Animals	
				No.[a]	%	No.[a]	%
2015	331	17176	-	59	17.8	739	4.3
2016	217	13894	78	78	35.9	1130	8.1
2017	483	22609	236	114	23.6	725	3.2
2018	465	20353	209	107	23.0	631	3.1
2019	587	26710	222	200	34.1	1095	4.1
2020	648	28616	205	227	35.0	899	3.1
Total tested	2731	129358		785		5219	
Total unique identity tested	1280	83602		442	34.5	5198	6.2

[a]No. of unique identity farms or animals tested or with positive results in that year; many farms and some animals were tested more than once per year; the same farm or animal can appear in more than one year; the total number of unique identity farms and animals are shown in the last row.

474; range 217–648) were tested per year during the 6 years of this study (Table 2).

A total of 83,602 individual animals were tested during the 6 years of the study with some of them tested more than once according to the BTEC unique identity animal numbers from 2015 to 2020. This number might underrepresent or overrepresent the total number of individual animals tested before 2018, because during this period a hard copy list of animal ID numbers to be tested was not taken on each farm test visit and no record of lost metal ear tags or replacement ear tag ID numbers was kept. It is also likely that there were missing hard copies of field sheets for 2018.

Central and Western Division were visited each year from 2015, while testing commenced in Northern division from 2017 and Eastern division in 2020, so that 2020 was the only year in which all 4 divisions were visited (Figure 3). All provinces were consistently tested in Central Division and in Western Division. For Northern Division, all provinces were included only in 2017 and 2020 compared to just one province out of four in Eastern Division.

The majority of animals tested were in Central Division with an average of 17,373 cattle tested per year between 2015 - 2020 (median 17,744; range 13,008–20,099). Then, in decreasing order of total number of animals tested were: Western Division (median 2,654; range 913–5,201) tested in 6 years, Northern Division (median 2,022; range 254–3,646) tested in 4 years, and the Eastern Division with only 104 animals tested in 2020 (Figure 3).

Coverage

From 2019 to 2020, based on the 2020 FAC, BTEC had a greater average coverage of dairy cattle (30.3%) than beef cattle (19.1%) across Fiji, and a greater average coverage of cattle in the Central Division (59.8%) compared to Western (8.0%), Northern (13.5%) and Eastern Divisions (2.5%) (Table 3). In Central Division, on average 72.5% of the dairy cattle were tested (Table 3). In 2020, a greater number of beef farms were tested than in previous years due to higher enrolment of new farms from this sector and extension of the BTEC program (Table 4).

Before 2015, holding facilities were not visited by BTEC, but during the study period and with the increased restrictions on movement of untested cattle, farmers reclaiming their cattle from holding facilities requested testing of their animals before return to their farm. The number of bTB tests conducted in holding facilities increased gradually from 2015 to 2019, but in 2020 the number of tests decreased because animals in these facilities were sent directly to the abattoir for slaughter (Table 4).

Detection of bTB nationally and by division and province

A total of 5,219 bTB positive tests were identified nationally from 2015 to 2020, corresponding to 5,198 unique identity animals (21 of which had multiple tests) from a total population of 83,602 (6.2%) cattle that were tested (Table 2). This represents an overall reactor rate or apparent prevalence of 6.2%. The reactors came from 442 unique identity farms, which represented 34.5% of the total of 1280 farms that were tested.

On average, 870 cattle (median 819; range 631–1130) from 131 farms (median 111; range 59–227) tested positive per year during the study. bTB positive cattle were identified in 3 of 4 geographic divisions, and in these 3 divisions testing was performed consistently over 4 or more consecutive years (Figure 3).

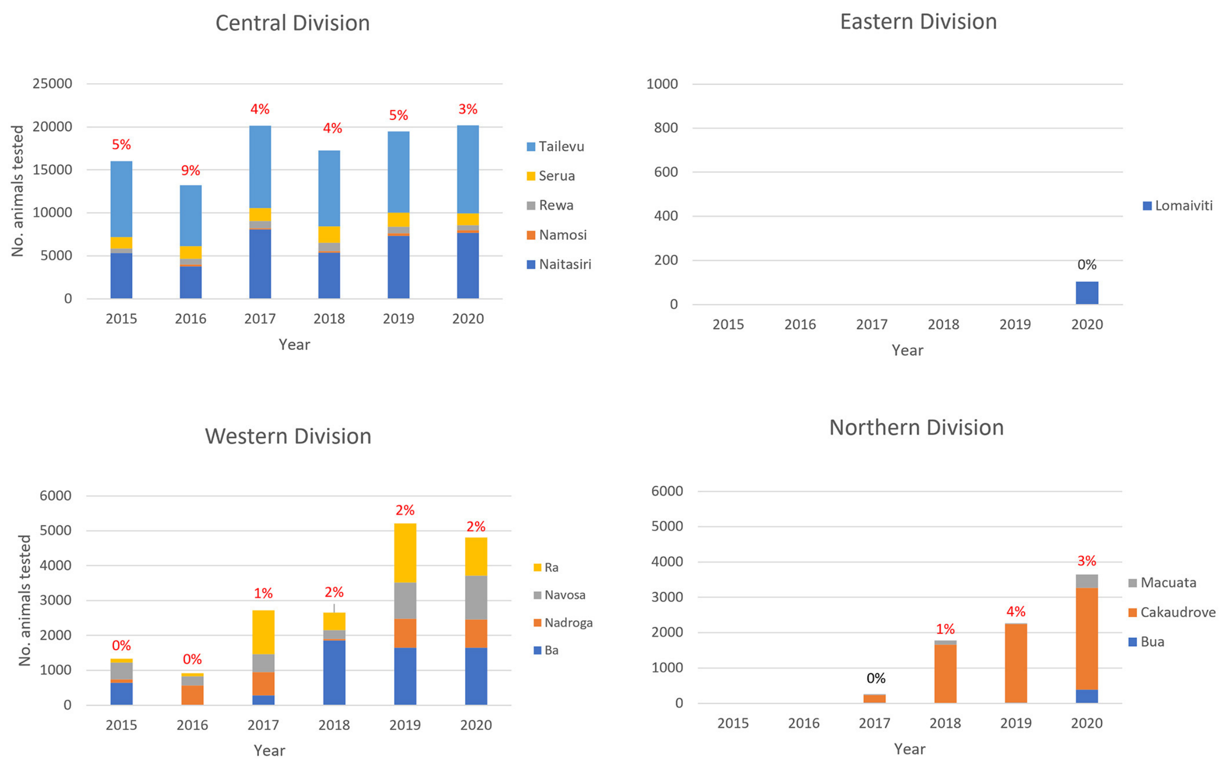

FIGURE 3
Total number of unique identity cattle tested each year by division and province in the Fiji BTEC program from 2015 to 2020. The percentage positive for bovine tuberculosis is shown above each bar.

TABLE 3 BTEC coverage of beef cattle, dairy cattle, and the total cattle population in Fiji by division in 2019 and 2020. All data were unique identity.

Year	Division	No. beef cattle			No. dairy cattle			Total cattle population[a]		
		Census	BTEC	Coverage (%)	Census	BTEC	Coverage (%)	Census	BTEC	Coverage (%)
2019	Central	12,924	5,235	40.5	20,093	14,163	70.5	33,017	19,350	58.6
	Western	40,244	4,181	10.4	22,310	650	2.9	62,554	5,201	8.3
	Northern	15,265	2,210	14.5	6,744	-	0.0	22,009	2,269	10.3
	Eastern	1,608	-	0.0	503	-	0.0	2,111	-	0.0
	Total	70,041	11,626[b]	16.6	49,650	14,813	29.8	119,691	26,820[b]	22.4
2020	Central	12,924	5,225	40.4	20,093	14,967	74.5	33,017	20,099	60.9
	Western	40,244	4,337	10.8	22,310	362	1.6	62,554	4,809	7.7
	Northern	15,265	3,611	23.7	6,744	-	0.0	22,009	3,646	16.6
	Eastern	1,608	104	6.5	503	-	0.0	2,111	104	4.9
	Total	70,041	13,277[b]	19.0	49,650	15,329	30.9	119,691	28,658[b]	23.9

[a]This total includes beef cattle, dairy cattle and a small number of cattle at holding facilities (n = 381 in 2019; n = 52 in 2020). [b]This total is the sum of the unique identity animals by division. Animals that moved between divisions in a calendar year and were tested in each division would be counted twice. Thus, these totals are slightly higher than in Tables 2, 4.

Central division

Between 2015 and 2020, a total of 4,724 unique identity cattle out of a total population of 64,663 (7.3%) that were tested were detected as bTB reactors in Central Division. They came from 336 unique identity farms, which represented 38.3% of the total of 877 unique identity farms that were tested.

For Central Division, testing was conducted each year in the 5 provinces (Naitasiri, Namosi, Serua, Tailevu, Rewa) (Supplementary Table 2). On average, a total of 791 cattle (median 721; range 576–1127) from 110 farms (median 95; range 56–178) tested positive to bTB each year during the study period.

TABLE 4 Beef farms, dairy farms and holding facilities: Number of tests, number of farms and animals tested for bTB by the Fiji BTEC program from 2015 to 2020.

Farm Type	Number of unique identity farms across the study period [b]	Year	Number of tests	Number of		Number of	Positive results			
							Farms		Animals	
				Farms tested	Animal tested	New farms	No.	%	No.	%
Beef	822	2015	2870	78	2860	-	9	11.5	31	1.1
		2016	3510	70	3101	55	18	25.7	165	5.3
		2017	9849	230	8013	184	36	15.7	106	1.3
		2018	7927	202	6946	148	41	20.3	133	1.9
		2019	13633	284	11606	176	78	27.5	413	3.6
		2020	17575	355	13270	185	113	31.8	386	2.9
Dairy	435	2015	16105	252	14434	-	50	19.8	708	4.9
		2016	13711	144	10901	21	60	41.7	966	8.9
		2017	18227	248	14844	50	77	31.0	619	4.2
		2018	17158	246	13650	48	63	25.6	491	3.6
		2019	22826	285	14808	43	117	41.1	674	4.6
		2020	27447	276	15327	23	113	40.9	512	3.3
Holding facilities [a]	23	2015	11	1	11	-	-	-	-	-
		2016	31	3	29	2	-	-	-	-
		2017	233	5	223	2	1	20.0	1	0.4
		2018	477	16	399	12	3	18.8	9	2.3
		2019	561	18	546	4	5	27.8	8	1.5
		2020	222	17	222	2	1	5.9	1	0.5

[a] Holding facility: a site designated for holding cattle for short periods of time such as impounded straying cattle with and without ear tags. [b] Total number of unique identity farms that were visited by BTEC from 2015 to 2020. In the columns to the right, data are the number of tests applied, the unique identity farms or animals tested, and the farms or animals with positive results in that year. Many farms and some animals were tested more than once per year; the same farm or animal can appear in more than one year, but within a year a farm or animal appears only once.

bTB positive cattle were detected consistently in all provinces, but particularly in Tailevu and Naitasiri, which had the most reactors during the study. In Tailevu, on average 554 cattle (median 495; range 433–791) from 51 farms (median 47; range 33–75) tested positive each year. In Naitasiri, on average 163 cattle (median 151; range 68–322) from 45 farms (median 37; range 19–79) tested positive to bTB each year. In Serua, on average 45 cattle (median 32; range 12–111) from 8 farms (median 7; range 2–16) tested positive to bTB each year. In Rewa, on average 27 cattle (median 29, range 1–58) from 5 farms (median 5, range 1–7) tested positive to bTB each year. In Namosi, on average 4 cattle (median 1, range 1–8) from one farm (median 1, range 1–3) were bTB positive each year.

Western division

Between 2015 and 2020, a total of 250 unique identity cattle out of a total population of 14,385 (1.7%) that were tested were detected as bTB reactors in Western Division. They came from 85 unique identity farms, which represented 24.6% of the total of 346 unique identity farms that were tested.

For Western Division, testing was conducted from 2015 to 2020 in the 4 provinces (Ba, Nadroga, Navosa and Ra) (Supplementary Table 3).

Northern division

Between 2017 and 2020, a total of 225 unique identity cattle out of a total population of 5,557 (4.1%) that were tested were detected as bTB reactors in Northern Division. They came from 21 unique identity farms, which represented 42.0% of the total of 50 unique identity farms that were tested.

For Northern Division, testing was conducted from 2017 to 2020 across its 3 provinces (Bua, Cakaudrove and Macuata) (Supplementary Table 4).

Eastern division

Cattle in the Eastern Division (Lomaiviti province) were tested only in 2020, with no bTB positives detected from among the 140 cattle tested.

TABLE 5 Number of farms tested by the BTEC program and proportion of bTB positive by farm type from 2015 to 2020 across the four Divisions of Fiji.

Division	Number of unique identity farms across the study period [a]	Year	Beef farms Total	Beef farms Positives (%)	Dairy farms Total	Dairy farms Positives (%)	Holding facilities Total	Holding facilities Positives (%)
Central	532 beef farms	2015	63	8 (12.7)	237	48 (20.3)	1	-
	339 dairy farms	2016	58	17 (29.3)	143	60 (42.0)	1	-
	6 holding facilities	2017	182	32 (17.6)	244	77 (31.6)	1	1 (100.0)
		2018	125	25 (20.0)	206	54 (26.2)	2	1 (50.0)
		2019	187	53 (28.3)	239	106 (44.4)	4	2 (50.0)
		2020	236	69 (29.2)	253	109 (43.1)	6	-
Western	238 beef farms	2015	15	1 (6.7)	15	2 (13.3)	-	-
	96 dairy farms	2016	12	1 (8.3)	1	-	2	-
	12 holding facilities	2017	42	4 (9.5)	4	-	4	-
		2018	68	13 (19.1)	40	9 (22.5)	10	1 (10.0)
		2019	89	23 (25.8)	46	11 (23.9)	10	1 (10.0)
		2020	75	27 (36.0)	23	4 (17.4)	8	1 (12.5)
Northern	45 beef farms	2015	-	-	-	-	-	-
	5 holding facilities	2016	-	-	-	-	-	-
		2017	6	-	-	-	-	-
		2018	9	3 (33.3)	-	-	4	1 (25.0)
		2019	8	2 (25.0)	-	-	4	2 (50.0)
		2020	37	17 (45.9)	-	-	3	-
Eastern	7 beef farms	2020	7	-	-	-	-	-

[a] Total number of unique identity farms visited by BTEC from 2015 to 2020. In the columns to the right, data are the farms tested with positive results in that year. Many farms were tested more than once per year; the same farm can appear in more than one year, but within a year a farm appears only once.

Detection of bTB by type of farm operation

Overall, between 2015 and 2020, bTB was detected on 24% of beef farms, 54% of dairy farms and 35% of holding facilities that were tested, with a significantly higher proportion of bTB positive dairy farms than beef farms ($P < 0.0001$). In beef farms, on average 206 cattle (median 149; range 31–413) from 49 farms (median 39; range 9–113) tested positive to bTB each year (Table 4). In dairy farms, on average 662 cattle (median 647; range 491– 966) from 80 farms (median 70; range 50–117) tested positive to bTB each year. On average 5 cattle (range 1–9) from 3 holding facilities (range 1–5) tested positive to bTB each year from 2017 to 2020 (Table 4).

From 2015 to 2020, the proportion of bTB positive beef farms was similar across Central Division and Western Division, ranging from around 10 to 30% each year (Table 5). For dairy farms, higher proportions of bTB infected farms were found each year in Central Division (range 20 to 44%) compared to Western division (range 13.3–24%). In Central Division, bTB positive cattle were detected in approximately 2 of 4 holding facilities each year from 2017 to 2020. In Western Division, in approximately 1 of 10 holding facilities contained bTB infected cattle from 2018 to 2020 while in Northern Division, approximately 2 of 4 holding facilities contained bTB infected cattle during that period (Table 5).

bTB on previously and newly enrolled farms

Previously untested farms were enrolled in the BTEC program each year. The newly enrolled beef farms with bTB positive animals were located across Western Division and Central Division from 2016 to 2020, and in Northern Division in 2018 and 2020. The newly enrolled dairy farms with bTB positive animals were found in Central Division from 2016 to 2020 and from 2018 onwards in Western Division (Figure 4).

Dairy farms

Prevalence

Between 2015 and 2020, a total of 3,956 unique identity cattle out of the total of 52,159 [7.6%, 95% CI (7.36, 7.81)] that were tested were detected as bTB reactors on dairy farms. They came

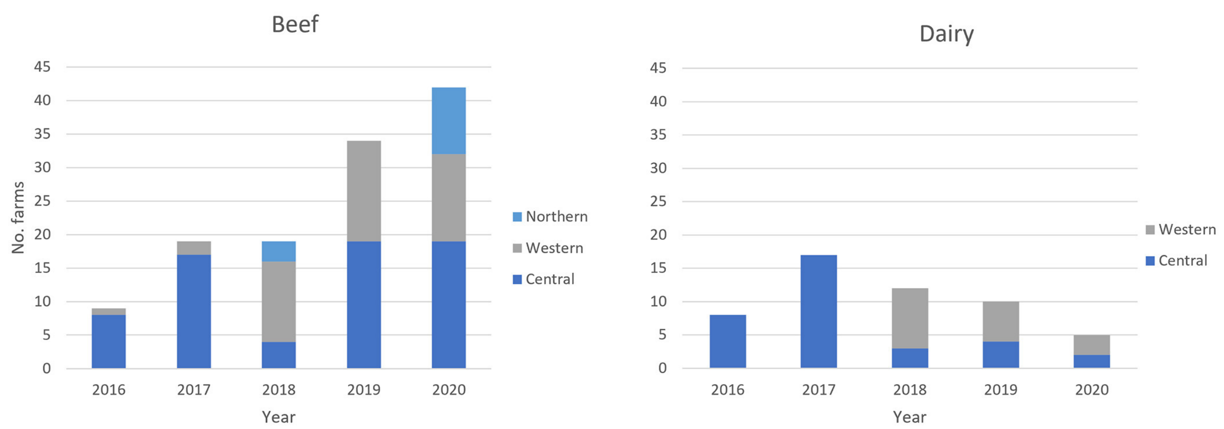

FIGURE 4
Geographic location of the bTB positive newly enrolled beef and dairy farms from 2016 to 2020.

from 234 unique identity dairy farms, which represented 53.8% of the total 435 unique identity farms that were tested.

Dairy farms were covered by the BTEC program only in Central Division and Western Division (Table 5). In Central Division, bTB positive cattle were found each year from 2015 to 2020 and in the Western Division in 2015, 2018, 2019 and 2020.

In Central Division, between 2015 and 2020, a total of 3,912 unique identity dairy cattle out of a total of 50,237 (7.79%) that were tested were detected as bTB reactors on dairy farms. They came from 209 unique identity dairy farms, which represented 61.65% of the total 339 unique identity farms that were tested. On average, a total of 76 farms (median 69; range 48–109) tested positive each year (Table 5).

In Western Division, between 2015 and 2020, a total of 44 unique identity dairy cattle out of a total of 1,985 (2.2%) that were tested were detected as bTB reactors on dairy farms. They came from 25 unique identity dairy farms, which represented 26.0% of the total 96 unique identity farms that were tested. On average, a total of 7 farms (median 7; range 2–11) tested positive each year (Table 5).

Detection of bTB by size of dairy farm operation and division

Overall, between 2015 and 2020, according to unique identity farm data, bTB was detected on 87.6% of commercial farms, 58.6% of semi-commercial farms and 40.2% of subsistence farms in Central Division. Overall, of the three sizes of farm operations, the proportion of bTB positive commercial farms was significantly higher ($P < 0.0001$) than the other sizes. For Central Division, from 2015 to 2020, on average, 559 cattle (median 530; range 324–667) from 41 commercial farms (median 40, range 28–58), 63 cattle (median 68; range 20–93) from 25 semi-commercial farms (median 21, range 12–44), and 33 cattle (median 18; range 8–113) from 9 subsistence farms (median 9, range 8–11) were detected bTB positive each year during the study (Table 6).

For Western Division, bTB was detected on 31.8% of semi-commercial farms and 22.4% of subsistence farm; no bTB reactors were detected on commercial farms (Figure 5).

Farm bTB status

In 2019 and 2020, only 21.3% (51/239) of the tested dairy farms maintained their clear status, another 8.4% (20/239) reverted to infected after one year or more of being bTB clear, and most farms remained infected during these 2 years. Five of the farms that reverted to infected status had untested cattle during the time that they were considered to be clear from bTB.

Untested animals

Untested animals were recorded only during 2019 and 2020. Before this, the farmer may have informed the BTEC team of missing animals but there was no way to capture the information. In 2019, the proportion of farms with untested animals in Central Division was higher in Naitasiri and Tailevu provinces with similar distribution across beef and dairy cattle (Table 7).

Missing animals

Missing animals were recorded only during 2019 and 2020. An increase in the number of reported missing animals occurred in 2020 across all farm sizes of operation.

TABLE 6 Dairy farms in Central division by operation size: Number of tests, number of farms and animals tested for bTB by the Fiji BTEC program from 2015 to 2020.

Operation size	Number of unique identity farms across the study period[a]	Year	Number of tests	Number of Farms tested	Number of Animal tested	Number of New farms	Number and percentage with positive results: Farms No.	Farms %	Animals No.	Animals %
Commercial	97	2015	11660	76	10236	-	28	36.8	667	6.5
≥ 41 cattle		2016	11351	58	8833	8	36	62.1	893	10.1
		2017	12816	77	10280	9	43	55.8	503	4.9
		2018	11743	71	9538	1	29	40.8	324	3.4
		2019	17147	82	10284	2	58	70.7	556	5.4
		2020	20736	84	10759	1	54	64.3	408	3.8
Semi-commercial	145	2015	3075	99	2994	-	12	12.1	20	0.7
≥ 16–≤40 cattle		2016	1886	53	1736	6	15	28.3	56	3.2
		2017	4326	108	3840	19	24	22.2	91	2.4
		2018	3724	91	3065	6	17	18.7	37	1.2
		2019	4338	111	3430	10	40	36.0	93	2.7
		2020	5367	116	3631	5	44	37.9	79	2.2
Subsistence	97	2015	956	62	952	-	8	12.9	18	1.9
≥ 1–≤15 cattle		2016	466	32	460	6	9	28.1	18	3.9
		2017	1026	59	913	18	10	16.9	25	2.7
		2018	925	44	759	6	8	18.2	113	14.9
		2019	687	46	570	1	8	17.4	8	1.4
		2020	977	53	682	4	11	20.8	18	2.6

[a]Total number of unique identity farms that were visited by BTEC from 2015 to 2020. In the columns to the right, data are the number of tests applied and the unique identity farms or animals tested or with positive results in that year. Many farms and some animals were tested more than once per year; the same farm or animal can appear in more than one year, but within a year a farm or animal appears only once.

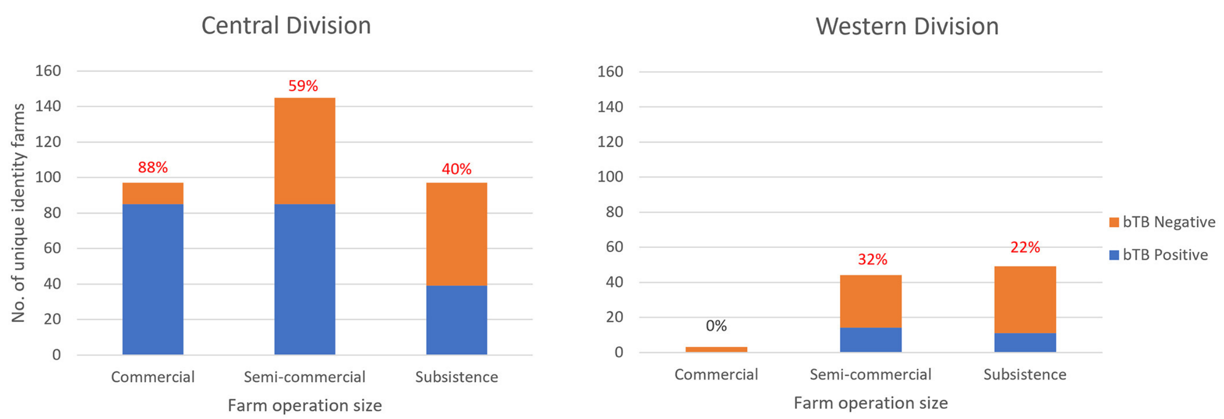

FIGURE 5
The number of bTB negative and positive unique identity farms classified by operation size, based on tests conducted by the Fiji BTEC program between 2015 and 2020. The % of farms with bTB reactors are shown above each bar.

TABLE 7 Proportion of beef and dairy farms in Central Division with untested animals in 2019 and 2020.

Division	Number of unique identity animals untested[a]	Province	Farms 2019						Farms 2020					
			Total No.		Farms with non-tested cattle[b]				Total No.		Farms with non-tested cattle[b]			
			Beef	Dairy	Beef	%	Dairy	%	Beef	Dairy	Beef	%	Dairy	%
Central	1561	Naitasiri	61	110	21	34.4	68	61.8	92	117	34	37.0	68	58.1
		Namosi	6	0	1	16.7	-	-	7	0	2	28.6	-	-
		Rewa	17	13	4	23.5	-	23.1	16	13	1	6.3	-	-
		Serua	38	15	3	7.9	9	60.0	35	16	13	37.1	9	56.3
		Tailevu	69	102	17	24.6	23	22.5	91	109	18	19.8	53	48.6

[a]Total number of unique identity animals untested from 2019 to 2020.
[b]Farms with at least one animal without a complete bTB test.

In 2019 and 2020, on average, the commercial farm category had the highest number of missing cattle, being 1,729 (21.2%) cattle from 67 (80.5%) farms compared to 895 (30.2%) from 79 (68.1%) semi-commercial farms and 167 (24.7%) from 34 (64.6%) subsistence farms (Supplementary Table 5).

bTB lesion detection at the abattoir

The highest numbers of reactors with visible lesions were found in 2015 and 2018 when there were 784 and 852 animals, respectively, while the lowest numbers of reactors with visible lesions were found in 2017 and 2020 when there were 250 and 186, respectively. The number of animals with NVL fluctuated considerably between years, ranging from 423 to 760. From 2018 to 2020, there was a decrease in the proportion of reactors with visible lesions compared to NVL (Figure 6).

bTB laboratory diagnostics

In 2018, the proportion of bovine tissue samples (visible lesions and NVL) sent to the laboratory that were presumptively positive for M. bovis by culture was 66.7%. In 2019 and 2020, only samples from NVL reactors were sent to Fiji Veterinary Laboratory for culture. The percentage of samples positive for bacterial isolation in Löwenstein-Jensen pyruvate agar was 90% (463/512) in 2019, and 68% (354/520) in 2020 (Supplementary Table 6). During those years, on average 79% were presumptively culture positive for *M. bovis*. In 2018, 66.2% of the cultures from the NVL reactors were positive, i.e., a lower percentage compared to 2019 and 2020 results. In 2019, 14 bacterial cultures in Löwenstein-Jensen pyruvate were submitted for *Mycobacterium* species identification by PCR and 92.8% were confirmed to be positive for *M. bovis*.

Animal movements

Movements from non-clear farms

To a farm in a different province

In 2019, most of the animals that were moved from non-clear farms to any other farm between different provinces were sent to Central Division. Ninety-five farms in 9 provinces and 3 divisions sent a total of 153 animals to 83 farms in Central Division. Eventually, three of these moved animals were identified to be bTB positive and slaughtered at FMIB Nasinu abattoir in Central Division (Table 8).

In 2020, there was an apparent reduction in such movements with only thirteen farms in 4 provinces and 2 divisions moving a total of 34 animals to 13 farms in Central Division (Table 8). Eventually, one of the moved animals tested positive and was sent to slaughter at FMIB Nasinu abattoir in Central Division.

In 2019, of the animals tested in each division, 0.8% of animals in Central Division, 0.8% in Western Division and 0.3% in Northern Division had been moved from non-clear farms in different provinces in the same or a different division. However, in 2020, such movements decreased to 0.2% from Central Division and 0.04% from Western Division (Table 8).

To a farm in the same province

In 2019, most of the animals that were moved from non-clear farms to any other farm in the same province occurred in Central Division. In total, 39 localities moved 263 animals across 102 farms in the Central division. Eventually, fifteen of these moved animals—two that tested positive to bTB before being moved and thirteen that subsequently tested positive to bTB—were sent slaughter at FMIB Nasinu abattoir in Central Division (Table 9). Of the animal population tested in each province, 2.1% from Naitasiri, 0.3% from Rewa, 0.2% from Serua and 1.1% from Tailevu province came from non-clear farms (Table 9).

In Western Division, in total, 6 localities across Ba, Nadroga and Ra provinces moved 7 animals to 6 farms. In Northern

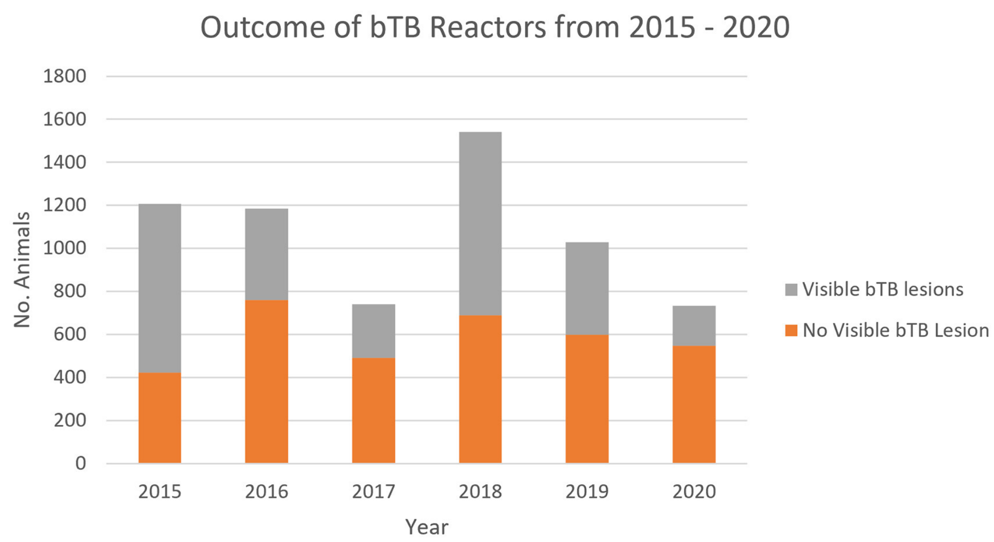

FIGURE 6
Post-mortem designation of all reactor animals sent to FMIB Nasinu abattoir.

Division, in total, 2 localities across Bua and Cakaudrove provinces moved 4 animals to 3 farms (Table 9).

In 2020, animal movements from non-clear farms within a province were registered only in Central Division. In total, 21 localities across Naitasiri and Tailevu provinces, moved 164 animals to 47 farms in Central Division. Of the animal population tested, 1.6% from Naitasiri and 0.4% from Tailevu were moved between non-clear farms. Eventually, four of these moved animals- two that tested positive to bTB before being moved, and two that subsequently tested positive to bTB—were sent slaughter at FMIB Nasinu abattoir in Central Division (Table 9).

Movements from clear farms

To a farm in a different province

In 2019, most of the animals moved from clear farms to any other farm between different provinces were sent to Central Division. Eleven farms in 5 provinces and 2 divisions sent a total of 17 animals to 14 farms in Central Division. None of these animals subsequently tested positive to bTB or were sent to slaughter at FMIB Nasinu abattoir (Table 8). In 2020, there was an apparent reduction in such movements with only 3 farms in 2 provinces and 1 division moving 9 animals to 4 farms in Central Division (Table 8). None of these animals subsequently tested positive to bTB or were sent to slaughter at FMIB Nasinu abattoir.

To a farm in the same province

In 2019, all the animal movements from clear farms to any other farm in the same province occurred in Central Division. In total, 18 localities moved 59 animals across 33 farms in Central Division. Eventually, 1 animal moved across Naitasiri province, subsequently tested positive to bTB, and was sent for slaughter at FMIB Nasinu abattoir in Central Division (Table 9). In 2020, 7 localities across Naitasiri and Tailevu provinces moved 128 animals to 11 farms. Eventually, two animals that were moved across Tailevu province subsequently tested positive to bTB and were sent for slaughter at FMIB Nasinu abattoir in Central Division (Table 9).

Discussion

According to Mussman (22), in less-economically developed countries that generally lack a mobile field service with trained veterinarians and auxiliaries, and that also often lack adequate diagnostic facilities, a disease control programme should have two stages. The first is short-term and includes the development of diagnostic and field services, and training of personnel to deal with diseases and implement control actions. The second is long-term and includes establishment of disease reporting systems and facilities for field surveys and use of the data from these for economic and epidemiological modeling to determine the benefits arising from the control program. To underwrite long-term implementation of an animal disease control program, there must be supporting legislation, political will to enforce the legislation and ongoing, dedicated allocation of government funds for the program. Given the lengthy incubation period for bTB, a control program must be sustained with strict adherence to protocols for an extended period and data be analyzed regularly in order to track progress for return on investment and

TABLE 8 Animal movements from non-clear farms to a farm in a different province and from clear farms to a farm in a different province during 2019–2020.

Movement origin		Movement destination				
Year	Division: Province	Division: Province	Animals tested[a]		Farms tested[a]	
			Total population	No. moved (%)	Total population	No. with movements (%)
Animal movements from non-clear farms						
2019	**Central:** Naitasiri, Namosi, Serua, Rewa, Tailevu. **Western:** Ba, Nadroga, Ra. **Northern:** Cakaudrove	**Central:** Naitasiri, Namosi, Serua, Rewa, Tailevu	19,350	153 (0.8)	430	83 (19.3)
	Central: Naitasiri, Serua, Tailevu. **Western:** Ba, Nadroga. **Northern:** Cakaudrove	**Western:** Ba, Nadroga, Navosa, Ra	5,201	44 (0.8)	145	24 (16.6)
	Central: Naitasiri, Tailevu. **Western:** Ra	**Northern:** Cakaudrove	2,269	6 (0.3)	12	2 (16.7)
2020	**Central:** Naitasiri, Serua, Tailevu. **Western:** Ba	**Central:** Naitasiri, Serua, Tailevu	20,099	34 (0.2)	495	13 (2.6)
	Central: Tailevu	**Western:** Ba	4,809	2 (0.04)	106	2 (1.9)
Animal movements from clear farms						
2019	**Central:** Naitasiri, Rewa, Serua Tailevu **Western:** Navosa	**Central:** Naitasiri, Namosi, Serua, Rewa, Tailevu	19,350	17 (0.1)	430	14 (19.3)
	Central: Namosi, Tailevu **Western:** Navosa	**Western:** Ba, Navosa, Ra	5,201	5 (0.1)	145	4 (2.8)
	Central: Tailevu	**Northern:** Cakaudrove	2,269	2 (0.1)	12	2 (16.7)
2020	**Central:** Naitasiri, Tailevu	**Central:** Naitasiri, Rewa, Tailevu	20,099	9 (0.04)	495	4 (0.8)

[a]Number of unique identity animals and farms involved in movements.

modification of diagnostic protocols as prevalence reduces. This level of sustained commitment is a serious challenge, particularly in a resource limited context (23–26). But it can lead to absence of bTB disease, as seen in the United States with implementation of control from 1917 achieving prevalence reduction from 5% to < 0.006% in 2011 (27) and even pathogen eradication, as achieved by the Australian control program over 27 years from 1970 to 1997 (6, 28).

Factors contributing to persistent bTB infections are common to all countries and revolve around there being persistent sources of *M. bovis*, but the details will vary from country to country. In Fiji, untested cattle and uncontrolled cattle movements were important contributors, whereas a wildlife reservoir has not yet been identified (29). Studies from Great Britain, the Republic of Ireland, Spain and Uruguay have associated these factors with bTB breakdowns (30–32). To significantly reduce bTB prevalence over time, South American countries such as Argentina, Brazil, Chile and Uruguay intensified their control measures for bTB based on surveillance at abattoirs, test-cull policy, bTB-free farm certification and disease notification (33). Most importantly, in these countries, a national livestock traceability system was implemented alongside these measures to control animal movements and to enable traceback from an abattoir to the original infected herd (33, 34). In countries such as England, the Republic of Ireland, New Zealand and USA, control measures for *M. bovis* reservoirs in wildlife have also been important to bring bTB under control (11, 35–37).

The long commitment of time required for the control bTB can be hindered because of inadequate financial support.

TABLE 9 Animal movements from non-clear farms to a farm in the same province and from clear farms to a farm in the same province during 2019-2020.

Year	Division	Province	Animals tested[a]		Farms tested[a]	
			Total no.	No. moved (%)	Total no.	No. moved (%)
Animal movements from non-clear farms						
2019	Central 263 animals, 102 farms, 39 localities	Naitasiri	7332	153 (2.1)	169	54 (32.0)
		Rewa	755	2 (0.3)	31	2 (6.5)
		Serua	1675	4 (0.2)	53	5 (9.4)
		Tailevu	9413	104 (1.1)	171	41 (24.0)
	Western 7 animals, 6 farms, 6 localities	Ba	1646	3 (0.2)	110	3 (2.7)
		Nadroga	838	1 (0.1)	10	1 (10.0)
		Ra	1693	3 (0.2)	11	2 (18.2)
	Northern 4 animals, 3 farms, 2 localities	Bua	23	1 (4.3)	4	1 (25.0)
		Cakaudrove	2215	3 (0.1)	5	2 (40.0)
2020	Central 164 animals, 47 farms, 21 localities	Naitasiri	7668	119 (1.6)	206	22 (10.7)
		Tailevu	10243	45 (0.4)	202	25 (12.4)
Animal movements from clear farms						
2019	Central 59 animals, 33 farms, 18 localities	Naitasiri	7332	16 (0.2)	169	11 (6.5)
		Tailevu	9413	43 (0.5)	171	22 (12.9)
2020	Central 128 animals, 11 farms, 7 localities	Naitasiri	7668	6 (0.1)	206	6 (2.9)
		Tailevu	10243	122 (1.2)	202	5 (2.5)

[a]Number of unique identity animals and farms involved in movements.

In South Africa, for example, re-prioritization of diseases and budget constraints led to a reduction in cattle testing which eventually produced re-emergence of bTB on commercial farms (24). Furthermore, an economic analysis of the cost of bTB-free certification of farms in Brazil reported that is feasible for larger-scale dairy farms, but for smaller scale, less efficient farmers, their inclusion in BTEC required targeted policies that compensated farmers for the additional costs (25). Thus, the lack of financial support compromises the coverage of the program. In contrast to countries like Fiji where participation in BTEC is mandatory, in some South American countries such as Ecuador, Peru, Bolivia, and Paraguay where bTB prevalence is high, regional campaigns are promoted based on the decisions of farmers to engage, but the bTB test is not mandatory for all cattle in the population. Therefore, the true burden of bTB disease remains unknown due to the lack of routine surveillance data, and it is therefore likely that *M. bovis* will persist in the population (38–40).

With respect to the objective of minimizing reservoirs of *M. bovis*, farmers in Fiji are fortunate that the government has supported BTEC testing, provides compensation for cattle that are culled and mandates testing of the national herd. These high-level objectives will require ongoing investment. In this study an objective analysis of data collected over 6 years was used to identify limitations and strengths in the bTB control program, and this led to important recommendations which are discussed below.

Limitations of the bTB control program in Fiji

Effective control of bTB requires identification of all infected animals. In Fiji, the coverage of the testing program was relatively low, with only 30% of dairy cattle and 20% of beef cattle being tested in 2019 and 2020. Coverage varied considerably depending on geographic region, was budget-dependent and had increased by 2018 as funding increased. The evidence suggested that bTB is highly prevalent across dairy farms and there was no visible trend downwards. Only one in five of the test negative dairy farms maintained its clear status over time, while most farms remained infected. Abattoir and laboratory data suggested that most bTB reactors in Fiji were truly infected with *M. bovis*.

This study provides insight on the factors contributing to persistent bTB infection on farms in Fiji: the bTB infection history of the farm, the size of the farm operation, the number of untested animals and missing animals and uncontrolled animal movements. Most of these factors were described in the previous BTEC retrospective study in Fiji which led to recommendations that informed the new BTEC strategy that was endorsed in 2018. Given the chronic nature of bTB and the long-term action needed to achieve control, this was expected, but with the recent improvements in data quality, we are now more confident in the designation of these factors.

Farms with only a relatively short period of clear status may still have residually-infected cattle that will contribute to farm bTB recurrence and to local/regional persistence of bTB. It is known that animals present on a farm during a bTB outbreak are likely later in their lives to become highly infectious (41, 42). In this study, 55% (11/20) of the dairy farms that changed from clear to infected status had been infected in earlier years of the study period. The future bTB risk of recurrence increases because of cattle-to-cattle transmission from residually-infected cattle in the herd or on neighboring farms, or due to transmission from the environment, wildlife, or humans, but the relevance of these factors needs to be determined for each farm (43, 44).

Consistent with previous findings in several countries (45, 46), in Fiji the risk of bTB was higher for larger farms and was consistently highest among commercial farms. Among these, some re-commenced bTB testing between 2017–2018 and retained reactors on farm until the start of the new compensation scheme in late 2018; in this context compensation had an unintended consequence. Compliance was higher during 2019 and 2020, with about 90% of reactor animals being sent to slaughter soon after detection.

In extensive farming situations in countries such as Fiji, if a whole herd test is not successfully completed, the untested animals can include some that remain a source of *M. bovis* infection for other animals on the farm. This is a problem in Fiji where many animals were identified as untested either because of an incomplete bTB test or due to not being presented by the farmer for testing. Many more cattle were identified as being missing; these were present in the dataset at one or more testing events but not later, without record of their death, slaughter, or sale. The possible reasons for missing animals include death, slaughter for consumption, sale, change to a new animal ID due to tag loss, incomplete muster or straying off the farm. Only some of the missing animals were likely to have permanently left the farm or been given a new ID; the rest probably remained on the farm and were untested.

Animal movements are known to be important for the spread of *M. bovis* in countries such as Great Britain, the Republic of Ireland and Uruguay (31, 47, 48) and were identified in this study in Fiji where they occurred across divisions and provinces from farms that did not have a bTB clear status. A proportion of these animals subsequently tested positive to bTB. Such movements need to be investigated. If movement restrictions are not imposed, export of infection to other farms will occur. A study by Clegg et al. (41) found that farms that experienced a bTB episode (2 or more reactors to skin test) and introduced animals early during the bTB episode were at significantly greater future bTB risk than farms where animals were introduced later (49).

Strengths of the bTB control program in Fiji

In Fiji, important improvements occurred in the bTB control program during 2014 to 2020, including for example, implementation of a new protocol for SID test reading and regular training of BTEC field staff, upskilling of meat inspectors, formulation of the new BTEC strategy for diagnostic and field services and endorsement of it by industry stakeholders (19). For the longer-term, Fiji has implemented *Bovibase*, the national recording system for the BTEC program and cattle production, which can provide the data for epidemiological and economic evaluations of progress. Strategically, the BTEC program was centralized to the national office in Suva from 2011 to 2019 to concentrate implementation on the dairy industry. Having achieved an increase in dairy farm participation, the BTEC program is now decentralized with the designated responsibility for conduct of the program returned to the MOA division offices in order to increase coverage, particularly of the beef industry. But with this, it is absolutely essential to maintain the level and proficiency of testing in Central Division in order to build on the gains achieved by the dairy industry and support individual farms to achieve and maintain clear status (50).

Whilst acknowledging these improvements, this study revealed several important aspects of Fiji bTB control that are critical to be acted upon if bTB prevalence is to be reduced; they require long-term commitment by the MOA, BAF and industry to achieve success. These are farmer engagement and compliance; implementation of animal movement regulations; consistent and harmonized use of *Bovibase* in order to implement, monitor and evaluate the control program; and continued collaborative research to inform refinement of diagnostic and control protocols. A third BTEC Stakeholder Forum is warranted now to engage all stakeholders in shared, evidence-based decision making on these issues. In addition to opening cattle trade opportunities between Fiji in the Pacific region, the eventual success of the BTEC program in Fiji will encourage neighboring Pacific countries to initiate bTB surveillance in their respective countries, learning from the strengths and limitations of the Fiji program.

Recommendations for the bTB control program in Fiji

Farmer engagement and compliance

Farmer engagement and compliance in an animal disease control program requires a combination of mechanisms to exist and to be applied consistently with support from government, industry and the community. These mechanisms relate to different factors that influence animal health behavior adoption such as external motivation provided by legislation with a formal requirement for participation and known consequences for non-compliance, and intrinsic motivation arising from sufficient knowledge to understand the risk posed to animal health and to their business, and confidence in ability to participate in and realize a benefit from the control program. Current legislation states the roles of specific government agencies in general animal disease control. It would be strengthened by inclusion of specific clauses specifying the roles of different government agencies and industry stakeholders in the continuous implementation of BTEC program as a One Health collaboration, with well-defined consequences for sector non-compliance.

In balance with legal requirements, government agencies need to provide extension services that educate and support farmers to understand bTB risk, to participate in testing and to adhere to cattle movement restrictions (discussed in next section). The important role of the MOA BTEC and BAF officers must be emphasized; in relation to provision of accurate information on bTB transmission and how regular whole herd testing and reactor removal along with cattle movement restrictions reduces transmission. Alongside the bTB control, the government extension officers need to provide services sought by farmers such as production advice and access to bTB-free replacement stock, and practical assistance to ensure testing of all cattle can occur and reactors be promptly sent to slaughter. For example, lack of on-farm animal handling facilities contributes to incomplete testing and farmers can be encouraged to setup simple holding yards to hold animals for further testing and hold reactors until sent to slaughter. This will assist in lowering the numbers of untested and missing animals. Additionally for compliant farmers with clear farms, it would be beneficial to provide a partial subsidy for fencing to minimize the risk of bTB recurrence posed by straying cattle (30).

To foster trust, respect, and farmer belief in the accuracy of information provided on bTB, MOA and BAF officers need to adhere to the SOPs and at the same time seek to develop a framework for communication with the farming community. It is important to examine the psychosocial factors that might influence the decision of farmers about compliance with the BTEC program. A study in Spain conducted interviews to identify the major themes related to how veterinarians and farmers were affected by their BTEC program. Participants mentioned that some of the weak points of the program were communication flow issues. This combined with the complexity of the bTB epidemiology and gaps in stakeholder knowledge contributed to disbelief and distrust in the bTB control program (51). A study by Robinson on qualitative narratives of bTB in Northern Ireland, concluded that farmers may resist rather than actively cooperate with the state because bTB is seen as just another of many farming life aspects demanding attention. Specters of global market forces, regulations, inspections, paperwork, bad weather, stress and disease overshadow and shape their attitudes and actions (52). In addition to enhancing the agenda at the BTEC forum, approaches to the communication issue in Fiji, could be the implementation of participatory studies, social capital studies, where the involvement of communities and development of trust is essential in defining and prioritizing the bTB problem, and in the development of solutions to service delivery, disease control options and surveillance (53, 54).

Regulation of cattle movement

The restriction of animal movement from non-clear farms needs to be ensured across Fiji because many such movements were discovered in this study. Continuous cooperation is needed from BAF to decrease animal movements from infected farms and clear farms that still have a high risk for bTB. Consistent and prompt implementation of penalties by BAF for non-compliance is necessary for bTB control in Fiji. Continuous enhancement of *Bovibase* to encourage the usage of its other components by the MOA Regulatory section, BAF, and FDL will allow for more effective monitoring of cattle movement, BTEC testing and herd production improvements in Fiji.

Data systems to monitor and evaluation

There was great complexity in the 2015 to 2020 BTEC data that were used in this study. To prepare the dataset for analysis required enormous effort and time due largely to the inconsistent implementation of a unique farm identification number across BTEC field testing data, abattoir and laboratory data. This delayed BTEC staff in uploading the source data to the new cloud-based platform *Bovibase*. However, the implementation of *Bovibase* has enabled the recording of data on BTEC activities with better identification and follow-up of participating farms and animals, recording of untested and missing animals, and stringent farm bTB status classification. The consistent use of *Bovibase* will play a critical role in harmonizing data between all cattle industry stakeholders and promote confidence in the information received about farms, geographic areas, and cattle movements (43, 55). Continued financial investment, development, and enhancement of *Bovibase* is required by the MOA as the lead agency for development of this national database. Stakeholders, particularly the meat and dairy inspector of the MOA Regulatory Section, BAF and Fiji Dairy Cooperative Ltd., must contribute to the maintenance of Bovibase through the provision of data, entering of data and financial subscriptions as pledged during its planning and development stages.

In terms of data quality, a critical need is the consistent use of a unique farm identifier based on geographical coordinates that is the sole identifier used by all agencies including the MOA Animal Health and Production Department, FMIB abattoirs, Fiji Veterinary Laboratory, the Biosecurity Authority of Fiji, FCDCL and Fiji Dairy Limited. This will enable seamless data sharing and provide timely and analysis-ready data, which is a prerequisite to provide evidence for the evaluation of BTEC policy outcomes for all stakeholders. On farm, individual animal identification also warrants improvement, and the use of RFID devices would provide an electronic id [e-id] linked with *Bovibase*, particularly for the dairy industry, to strengthen the determination of bTB farm status and achieve reliable animal traceability.

Other necessary enhancements are the timely encoding of BTEC field sheets into *Bovibase*, the incorporation of abattoir data for the update of animal status on slaughter to improve the accuracy of missing animal numbers and the bTB status of animals identified at abattoir slaughter inspection to contribute to farm status determination. In addition, an agreed protocol for data management and analysis is essential to enable consistent reporting. Reports should be automatically produced on a set schedule, such as weekly reports for farm field schedules, monthly reports of test results and updated clear farm status list, and quarterly reports for operational and budget purposes. Ideally the MOA field epidemiologists or committed senior officers would liaise with the *Bovibase* developer to refine the processes for data entry and data analysis.

Developing a dedicated research program funded by the government and the cattle industry in collaboration with international agencies will have the benefit of capacity building while providing important evidence to inform decision making on diagnostic and control protocols, and evaluation of the cost-benefit of implementation of bTB control at industry and farm levels. Immediate activities to enhance diagnostic capability and to address the presence of residual infected cattle, could include collaborations in repeating testing over time using SID, enhancing microbiological culture methods, introducing procedures for cell-mediated immune assays (interferon gamma release assay) to be evaluated alongside the SID test and establishing proficiency for PCR testing in-country at the Fiji Veterinary Laboratory. Moving beyond presumptive *M. bovis* culture positive status to confirmed status, discriminating *Mycobacterium* species recovered in culture, ruling out *M. tuberculosis* and strain typing for epidemiological tracing are important objectives. Given the importance of the SID for identification of bTB, data are needed from well-designed research trials to estimate the sensitivity and specificity of the SID test on farms with different bTB prevalence levels in Fiji, which would utilize gross necropsy findings from the abattoir and microbiology/PCR test results from the laboratory as independent tests for bTB (56). Maintenance of accurate records in *Bovibase* over time will enable advanced analyses such as spatio-temporal models of bovine tuberculosis in the cattle population and social network analysis (SNA) to characterize patterns of cattle movement and quantify the role of high-risk farms. Research objectives should be a topic for a 3rd BTEC Forum discussion with all stakeholders.

Conclusion

Relative to the stages of control reached in the decades-long bTB control programs in high-income countries, Fiji is in the early part of its control efforts. The program has been progressively improved and protocols have been enhanced based on technical analysis and stakeholder review. Reflecting this early phase, and consistent with the available financial and human resources, testing for bTB did not reach the majority of cattle in Fiji during the study period. Testing revealed that bTB was highly prevalent, with about 8% of dairy cattle and 62% of dairy farms infected. Against a high baseline prevalence of bTB, there was no visible downward trend in bTB apparent prevalence over 6 years. The factors contributing to this situation were objectively determined to be persistent infections, which were partly due to the significant number of untested animals and uncontrolled animal movements, and the particular importance of large farms. Data management was critical in the control program and is still evolving. There was complexity in the datasets that were compiled in this study, the lack of a consistent unique farm identification number and individual animal identification problems being responsible for time lost in correlating field-testing data, abattoir and laboratory data. However, the implementation, enhancement, and consistent use of *Bovibase* will play a critical role in harmonizing data. bTB remains a serious concern for the Fiji dairy industry and a public health risk for those that consume unpasteurized milk; there is value in One Health research on zoonotic bTB in high-risk communities, to support the human TB control program in Fiji. Implementation of a control strategy targeting the main contributors to bTB persistence that were identified in this study, along with maintenance of a comprehensive reporting and traceability system, industry awareness, and government support, will enable more farms to achieve and maintain a bTB clear status. Capacity building through scientific research that provides data to underpin policy and procedures in BTEC will be required. As in all countries, control of bTB in Fiji is a long-term objective that must have multiple stakeholder engagement and regular review to measure success.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions. The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author. Requests to access these datasets should be directed to jenny-ann.toribio@sydney.edu.au.

Author contributions

The proposal for this collaborative research was developed by J-AT, AG, EB, and RW. EB, SS, and VS facilitated collation, encoding and validation of field data and provided local documents for referencing. AG constructed and cleaned the dataset for analysis then performed all analysis and interpretation of results. EB and AR provided information about Bovibase contributing to validation of cleaned data and interpretation of the results. J-AT and RW supervised the analysis, presentation of the results and documentation of the outcomes and findings of the study. All authors contributed to the content and the preparation of this manuscript.

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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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2022.972120/full#supplementary-material

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Analysis of dairy cattle movements in the northern region of Thailand

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Dairy farming in northern Thailand is expanding, with dairy cattle populations increasing up to 8% per year. In addition, disease outbreaks frequently occur in this region, especially foot-and-mouth disease and bovine tuberculosis. Our goal was to quantify the underlying pattern of dairy cattle movements in the context of infectious disease surveillance and control as movements have been identified as risk factors for several infectious diseases. Movements at district levels within the northern region and between the northern and other regions from 2010 to 2017 were recorded by the Department of Livestock Development. Analyzed data included origin, destination, date and purpose of the movement, type of premise of origin and destination, and type and number of moved cattle. Social network analysis was performed to demonstrate patterns of dairy cattle movement within and between regions. The total numbers of movements and moved animals were 3,906 and 180,305, respectively. Decreasing trends in both the number of cattle moved and the number of movements were observed from 2010 to 2016, with increases in 2017. The majority (98%) of the animals moved were male dairy calves, followed by dairy cows (1.7%). The main purpose of the movements was for slaughter (96.3%). Most movements (67.4%) were shipments from central to northern regions, involving 87.1% of cattle moved. By contrast, 56% of the movements for growing and selling purposes occurred within the northern region, commonly involving dairy cows. Constructed movement networks showed heterogeneity of connections among districts. Of 110 districts, 28 were found to be influential to the movement networks, among which 11 districts showed high centrality measures in multiple networks stratified for movement purposes and regions, including eight districts in the northern and one district in each of the central, eastern, and lower northeastern regions of Thailand. These districts were more highly connected than others in the movement network, which may be important for disease transmission, surveillance, and control.

KEYWORDS

dairy cattle, movement, network, social network analysis, northern Thailand

Introduction

Milk production is projected to be the fastest growing agricultural commodity from 2021 to 2030. In March 2022, the international dairy prices marked a 24% increase compared to the same month last year (1). These persistent upward trends are driven by Asian countries, fulfilling almost one-third of the cow's milk production share globally. Behind these numbers, there are smallholder dairy producers, owning 1–5 animals each, which accounted for nearly 80% of milk production in Asia (2). Over 52% of these producers rely on their dairy business as the sole source of income. Livestock infectious disease is not only a direct threat to their livelihood but also an integral part of social order and stability for many developing countries (3).

A major challenge to disease surveillance and control among dairy production is the lack of documentation on animal movement, defined as the transportation of animals among various locations, such as breeding herds, feeding locations, markets, and slaughterhouses. Epidemiological examinations of animal movement data are typically carried out with social network analysis (SNA), a process of investigating interactions among members of a population through the graph theory (4). Combined with incidence data and sequencing data, foot-and-mouth disease (FMD) virus, bovine tuberculosis (bTB), and Johne's disease, among others, quantifications of cattle movement networks can (1) explain between-herd disease transmissions during outbreaks; (2) investigate multi-species transmissions in wildlife reservoirs; and (3) identify hot spots with an increased risk of infection for a cost-effective targeted surveillance (5–7).

Dairy farming in northern Thailand is expanding, with dairy cattle populations increasing up to 8% per year (8). In addition, disease outbreaks frequently occur in this region, especially FMD (9) and bTB (10, 11). Previously, we collected bTB case data from dairy-intensive areas such as Chiang Mai and Chiang Rai provinces of northern Thailand and found that farms importing cows from dealers in central Thailand had two times higher risk of infection than farms that purchased from other regions (11). Moreover, purchases made through dealers were associated with four times the higher risk of infection than purchases made directly between farms. These factors indicate the importance of connections between components of a network, such as farmers and dealers, in disease transmission, and the need to characterize these patterns to facilitate the development of control programs.

The objective of this study was to describe patterns of dairy cattle movements in the northern region of Thailand. This descriptive analysis can provide analytical context to identify key areas or districts with a high potential for disease transmission among dairy cattle in the northern region of Thailand and the surrounding regions where movement data are not available.

Materials and methods

Data of dairy cattle movement

All data involving dairy cattle that moved between districts within eight provinces in the northern regions of Thailand from 2010 to 2017 were obtained through collaboration with the Department of Livestock Development (DLD) in Thailand. The collected information included the date of movement, origin and destination locations (at district level), number and type of cattle moved, type of destination, and primary purpose of movement. In Thailand, the geographic hierarchy, ranked by specificity, includes regions, provinces, districts, and farms within districts. Specifically, the cattle movement dataset contains all legal movements that crossed a district border during the study period, including (1) from outside of the northern region of Thailand (including the lower north, central, east, lower northeast, upper northeast, and west regions of Thailand) to the northern region (Figure 1); and (2) within the northern region of Thailand (provinces of Chiang Rai (CR), Chiang Mai (CM), Lamphun (LP), Lampang (LPA), Mae Hong Son (MS), Nan (NA), Phrae (PR), and Phayao (PY)). Cattle movements within districts were not available. A complete list of districts, provinces, and regions is provided in Supplementary Table 2.

Data analysis

The analysis was carried out at two levels: regions and districts. The number of movements was summarized by year, the type of dairy cattle transported, the purpose of movement, and the premise type of the origin and destination. In addition, an identical analysis was performed on the subset of data that were non-abattoir movements as these movements might be associated with a greater risk of disease transmission between farms. Types of transported cattle included (1) bull, (2) dairy calf with no recorded sex, (3) female dairy calf, (4) male dairy calf, and (5) dairy cows. Primary purposes of the movement included (1) slaughtering, (2) growing, (3) selling, (4) export, and (5) semen collection. Types of premises of the origin and destination included (1) abattoir, (2) house, (3) farm, and (4) others, including market, government office, academic institute, dairy cooperative, private company, quarantine office, and temple. In many cases in Thailand, "farm" where the animals are kept and "house" where the farm owners live are at the same address. Because of the ambiguity of these two terms, data referring to these two terms were pooled together for analysis.

FIGURE 1
Map of Thailand illustrating the geographical distribution of the study area. Data used for the analysis involved the registered movement of dairy cattle across 110 districts from 32 of 77 provinces in seven of the nine regions of Thailand, including north, lower north, central, west, east, lower northeast, and upper northeast.

TABLE 1 Overall trend of the number of dairy cattle moved (animal head) and the number of dairy cattle movements (frequency) between districts in northern Thailand from 2010 to 2017.

Number	2010	2011	2012	2013	2014	2015	2016	2017	Total
Cattle moved	42,124 (23.4%)	34,589 (19.2%)	35,455 (19.7%)	23,796 (13.2%)	14,802 (8.2%)	10,454 (5.8%)	8,852 (4.9%)	10,233 (5.7%)	180,305 (100%)
Movements	832 (21.3%)	562 (14.4%)	554 (14.2%)	428 (11.0%)	339 (8.7%)	310 (7.9%)	300 (7.7%)	581 (14.9%)	3,906 (100%)

TABLE 2 Distribution of dairy cattle moved (animal head) and movements (frequency) between districts in northern Thailand from 2010 to 2017 stratified by month.

Month	Movements	Cattle moved
January	327 (8.37%)	15,355 (8.52%)
February	294 (7.53%)	14,239 (7.90%)
March	349 (8.93%)	15,854 (8.79%)
April	354 (9.06%)	17,712 (9.82%)
May	291 (7.45%)	14,300 (7.93%)
June	251 (6.43%)	10,419 (5.78%)
July	295 (7.55%)	12,709 (7.05%)
August	307 (7.86%)	14,212 (7.88%)
September	341 (8.73%)	16,784 (9.31%)
October	361 (9.24%)	17,263 (9.57%)
November	331 (8.47%)	15,319 (8.50%)
December	405 (10.37%)	16,139 (8.95%)
Total	3,906 (100.00%)	180,305 (100.00%)

Social network analysis was performed to characterize the movements of dairy cattle in northern Thailand. The analysis was stratified according to the primary purpose of the movement. Identifying influential districts associated with different purposes (i.e., growing, selling, or slaughtering) is essential for disease control surveillance because when an outbreak was detected in a slaughterhouse or a market, a purpose-specific network can be used for contact tracing to identify the source of infection as well as other locations exposed to transmission. In addition, not all documentation is perfect, especially for smallholder dairy producers with backyard farms. For example, documentation is better for abattoir movements to slaughterhouses than growing or selling movements to nearby farms. In addition, a previous study revealing that purchasing dairy cattle from the central region was a significant factor for bTB in dairy cattle farms in northern Thailand (11), indicating the importance of dairy cattle movements from different geographical regions for the disease outbreak. Therefore, the purpose-specific and region-specific networks can reveal detailed connections, which may be otherwise masked by the full network.

Given these justifications, a total of six north-centric weighted and directed networks were constructed: (1) a full network; (2) two purpose-specific networks including (a) growing and selling and (b) slaughtering; and (3) three region-specific networks including (a) movement from the central region to the northern region, (b) movement within the northern region, and (c) movement from other regions to the northern region. In each network, the nodes were districts, and the edges with directions were animal movements between the originating districts and the destination districts. The weights referred to the number of animals transported. The network analyses were performed using R version 4.2.0 with the package "igraph" version 1.3.1. A reproducible notebook including a full list of packages used are accessible at https://github.com/yyw-informatics/thailand_movement_network_analysis.

To capture the overall network structure, global metrics including density, mean path length, and transitivity were calculated. *Density* was measured by the ratio between observed movements and total possible movements among districts. Networks with high density are expected to have increased susceptibility in disease transmission. Networks with shorter *mean path length* (where the pathlength is the number of districts that must be passed through to connect any two districts in the network) could be an indication of a faster spread of infection. In addition, to identify interconnected clusters of districts, the network *transitivity*, defined as the ratio between the observed number of closed triplets and the total possible number of closed triplets, was calculated.

Network metrics were calculated to identify districts either with high movement frequency or positioned at important locations within the network that could disproportionately influence the spread between other districts, for example, bridging separated districts. Such districts were considered influential hot spots as they were expected to have a higher probability of becoming infected and transmitting diseases than other districts in the network. For each network, local metrics, including degree centrality, eigen centrality, strength, betweenness, closeness, and reciprocity were calculated to describe district-level connectivity and identify influential districts with an increased risk of infection and transmission.

- *Degree centrality* measured the number of direct connections held by each district, with in-degree referring to the number of inbound movements and out-degree referring to the number of outbound movements. *Eigen centrality* extended the degree centrality by calculating

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TABLE 3 Distribution of the different cattle types moved (animal head) between districts in northern Thailand from 2010 to 2017.

	2010	2011	2012	2013	2014	2015	2016	2017	Total
Bull	21 (0%)	1 (0%)	1 (0%)	12 (0%)	28 (0.2%)	9 (0.1%)	9 (0.1%)	187 (1.8%)	268 (0.1%)
Dairy calf [a]	80 (0.2%)	32 (0.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	112 (0.1%)
Female dairy calf	41 (0.1%)	27 (0.1%)	19 (0%)	55 (0.2%)	17 (0.1%)	39 (0.4%)	13 (0.1%)	8 (0.1%)	219 (0.1%)
Male dairy calf	41,071 (97.3%)	34,018 (98.5%)	35,128 (99.2%)	23,578 (99.1%)	14,471 (97.8%)	10,272 (98.2%)	8,792 (99.3%)	9,330 (91.2%)	176,660 (98.0%)
Dairy cow	991 (2.3%)	463 (1.3%)	275 (0.8%)	151 (0.6%)	286 (1.9%)	134 (1.3%)	38 (0.4%)	708 (6.9%)	3,046 (1.7%)
Total	42,204 (100%)	34,541 (100%)	35,423 (100%)	23,796 (100%)	14,802 (100%)	10,454 (100%)	8,852 (100%)	10,233 (100%)	180,305 (100%)

[a] Number of dairy calves with no specific gender recorded.

the second-order connections of districts. Districts with a high degree or high Eigen centrality indicated an increased frequency of movement, which may indicate an increased risk of disease exposure and onward transmission to other districts.

- As the number of animals associated with each movement was recorded in the data, *strength* was measured by summing up the weights associated with each edge. Districts with high strength might not have a high degree, but they could be associated with an increased probability of spreading infectious diseases due to the large volume of animals transported.
- *Betweenness centrality* measures the number of edges that traverse a district through the shortest path between each pair of districts. It measures the bridging effect of each district in the network. Districts with high betweenness might not have high frequencies of animal movement, but they acted like links between otherwise disconnected districts.
- *Closeness centrality* is the mean of distances of the shortest path between each pair of districts. It measures the extent to which a district is in the central position of a movement network. Districts with high closeness were expected to be well-connected with other districts in the network; hence, these districts have the potential to facilitate "super-spreading" transmissions in a star-like network.
- *Reciprocity* measures the likelihood of districts to be mutually connected. During outbreak investigations, districts with reciprocal movements suggested that both districts can be the source and destination, thus indicating an increased risk of infection and transmission.

Finally, districts were ranked based on the metrics discussed previously for each of the six networks. Using each of the centrality metrics, influential districts were selected if their centrality measures had exceeded the mean plus two standard deviations for that metric in the corresponding network. These districts either had high movement frequency or were essentially located, which can influence the flow of movement among separated clusters of districts. These districts were expected to be associated with an increased risk of infection and disease transmission. The results of these selected districts were first visualized in a heatmap showing the following information: (1) For *which network metric(s)*, the district had significant values? This information allowed evaluation of the role of the district in the corresponding network. For example, if the betweenness was high, then the district was bridging multiple separated districts. If the degree was high, then the district had a high frequency of movement. (2) In *how many network(s)* (of the six evaluated) the metric was found to be significant? For example, if a district with a high degree was found in multiple networks, then the selected district was likely to be important. (3) In *which network(s)* (of the six evaluated) the district was found to have significant

TABLE 4 Number of dairy cattle moved (animal head) and movements (frequency) between districts in northern Thailand for different primary purposes from 2010 to 2017.

Purpose	Cattle (%)	Movements (%)	2010	2011	2012	2013	2014	2015	2016	2017
Slaughtering	173,552 (96.3%)	3,195 (81.8%)	687 (82.6%)	497 (88.4%)	511 (92.2%)	389 (90.9%)	307 (90.6%)	263 (84.8%)	233 (77.7%)	308 (53%)
Growing	5,229 (2.9%)	484 (12.4%)	101 (12.1%)	56 (10%)	36 (6.5%)	36 (8.4%)	28 (8.3%)	45 (14.5%)	51 (17%)	131 (22.5%)
Selling	1,500 (0.8%)	225 (5.8%)	44 (5.3%)	9 (1.6%)	7 (1.3%)	3 (0.7%)	4 (1.2%)	2 (0.6%)	16 (5.3%)	140 (24.1%)
Export	20 (0%)	1 (0%)	0%	0%	0%	0%	0%	0%	0%	1 (0.2%)
Semen collection	4 (0%)	1 (0%)	0%	0%	0%	0%	0%	0%	0%	1 (0.2%)
Total	180,305	3,906	832	562	554	428	339	310	300	581

metrics? This information allowed the discovery of districts that were influential to certain stratified networks, such as the growing and selling network where the movement frequency was much less than the slaughtering network but covered much more districts, which would increase the probability of disease infection and transmission. Geographical maps showing these influential districts and the movement in their corresponding networks were used to demonstrate the spatial distance between these districts. Maps were created using the R package "ggmap" version 3.0.0 through queries of Google Maps.

Results

Descriptive summary

Movement by year

Data of dairy cattle movements in 110 districts of 32 provinces across seven regions in Thailand were included for the analysis (Figure 1). In total, 3,906 movements were documented, and 180,305 animals were moved (Table 1). Both numbers decreased consistently from 2010 to 2016, starting from 42,124 animals with 832 movements in 2010 to 8,852 with 300 movements in 2016. The only exception was in 2017, when the movements nearly doubled, although the number of animals moved was consistent with the previous 2 years. No apparent seasonality was observed across 12 months with the lowest numbers reported in June (Table 2).

Movement by cattle type

The animals moved were predominantly male dairy calves (98.0%, Table 3). The number of male dairy calf movements decreased during this time, from over 41,000 in 2010 to about 9,000 in 2017. The second most common cattle type moved was dairy cows (3,009 animals). The overall trend was decreasing for most animal types, except for bulls and dairy cows, both of which decreased from 2010 to 2016 and increased from 2016 to 2017.

Movement by purpose

The main primary purpose of the movement was slaughter, which accounted for 82.0% of all movements, contributing 96.3% of all cattle moved, followed by growing (12.2% of movements) and selling (5.7% of movements), as shown in Table 4. When evaluated by year (Table 4), slaughter accounted for at least 78% of cattle movements each year until 2016. In 2017, there was a different pattern of growing, selling, and slaughter, with 22, 24, and 53%, respectively.

Movement by destination

Abattoir was the most common destination, accounting for 77.4% of cattle moved (Table 5). The second most common destination was house or farm, accounting for 30.4% of movements and 22.4% of cattle moved. Across 8 years of study, the most frequent destination was the abattoir, except for 2010, followed by house or farm (Table 5). A comparison between the destination and the purpose of movements (Table 4) revealed that a total of 34,673 animals were moved for slaughtering purposes to non-abattoir premises.

Considering the movements to abattoirs as terminal movements of live animals, which are of less importance for some disease transmission, the movements to abattoirs were filtered out and reanalyzed to reveal the pattern of dairy cattle movements to other types of destinations. Among non-abattoir premises as destinations, house or farm was the predominant destination of movements, contributing 80.6 to 97.4% of these movements. House or farm also contributed over 76% of cattle moved to locations other than abattoirs (Table 6). In many of these non-abattoir movements, dairy cows were the most common cattle type moved (depending on the year, 12.3 to 78.9% of movements and 2.1 to 90.8% of cattle moved). The numbers of farms and houses associated with the dairy cattle movement in each district are provided in Supplementary Tables 1, 3, respectively.

Movement frequency across regions

In northern Thailand, the majority of the movements (67.3%) was from the central region, accounting for 87.1% of cattle moved into northern Thailand from elsewhere (Table 7). Data showed that animals involved in these movements were predominantly male dairy calves for slaughtering, which totaled

TABLE 5 Number of dairy cattle movements (frequency) and dairy cattle moved (animal head) between districts in northern Thailand to different premises of destination from 2010 to 2017.

Premises of destination	Number	2010	2011	2012	2013	2014	2015	2016	2017	Total
Abattoir	Cattle	5,843 (13.9%)	34,008 (98.3%)	34,967 (98.6%)	22,989 (96.6%)	14,475 (97.8%)	9,922 (94.9%)	8,143 (92.0%)	9,116 (89.1%)	139,463 (77.3%)
	Movements	138 (16.6%)	498 (88.6%)	516 (93.1%)	389 (90.9%)	307 (90.6%)	263 (84.8%)	233 (77.7%)	306 (52.7%)	2,650 (67.8%)
Farm/House	Cattle No.	36,090 (85.7%)	550 (1.6%)	485 (1.4%)	787 (3.3%)	252 (1.7%)	460 (4.4%)	703 (7.9%)	1,078 (10.5%)	40,405 (22.4%)
	Movements	663 (79.7%)	60 (10.7%)	37 (6.7%)	36 (8.4%)	26 (7.7%)	40 (12.9%)	64 (21.3%)	265 (45.6%)	1,191 (30.5%)
Others	Cattle No.	191 (0.4%)	31 (0.1%)	3 (0%)	20 (0.1%)	75 (0.5%)	72 (0.7%)	6 (0.1%)	39 (0.4%)	437 (0.2%)
	Movements	31 (3.7%)	4 (0.7%)	1 (0.2%)	3 (0.7%)	6 (1.8%)	7 (2.3%)	3 (1.0%)	10 (1.7%)	65 (1.7%)
Total	Cattle No.	42,124	34,589	35,455	23,796	14,802	10,454	8,852	10,233	180,305
	Movements	832	562	554	428	339	310	300	581	3,906

155,802 animals and accounted for 99.2% of all animals transported from central to northern Thailand.

Movement within northern Thailand

In 2017, the number of movements within the northern region was nearly 10 times higher than that the previous year, and it has exceeded the total from the central region for the first time since 2010 (Supplementary Figure 1). Over half of the movements were the movements of dairy cows (56%) for growing and selling purposes (69%) to house, which accounted for 63% of the documented destinations.

Network analysis on a district level

The heterogeneous distribution of network connectivity among districts

Overall, most districts were active as nearly 70% had more than one movement. However, the level of connectivity was different. Graph-level network metrics over the study years, from 2010 to 2017, are summarized in Table 8. No significant temporal signal was observed. On the district level, the distributions of most local network metrics were highly skewed (Supplementary Figure 2), suggesting the presence of several highly connected districts. For example, in the full network, the mean degree was 70.38, with a median of 4, due to four districts having more than 500 movements. Similar distributions were observed for other metrics including eigen centrality, betweenness, closeness, and strength.

The contrasting characteristics of networks stratified for movement purposes

Networks stratified for different movement purposes, either slaughtering (SA network) or growing and selling (GS network), showed contrasting patterns. Because of the large volume of animals and movements, the SA network had a much higher level of strength (mean = 152.14) than the GS network (mean = 13.13), as seen in Table 9. The GS network contained over 97% of districts in the full network (108 of 111 nodes), representing higher district coverage than the SA network (42 nodes). As seen in Figure 4, when simplifying the movement edges between two districts into a single edge, the SA network had fewer edges than the GS network Consequently, compared with the SA network, the GS network had higher betweenness (betweenness $_{GS=}$ 50.67 vs. betweenness $_{SA}$ 3.69, respectively) and longer average path length (length $_{GS=}$ 3.2 vs. length $_{SA}$ = 2.12, respectively).

TABLE 6 Number of dairy cows moved (animal head) among the total cattle moved (animal head) and their corresponding movements (frequency) by types of premises of destination other than abattoirs in northern Thailand from 2010 to 2017.

Premises of destination	Number of animals and movements	2010	2011	2012	2013	2014	2015	2016	2017	Total
Farm/	Cattle	36,090	550	485	787	252	460	703	1,078	40,405
House	Dairy cow	755	429	219	132	227	68	29	505	2,364
	% of dairy cow	2.1	78.0	45.2	16.8	90.1	14.8	4.1	46.8	5.9
	Movements	663	60	37	36	26	40	64	265	1,191
	Dairy cow movements	100	47	26	17	18	14	8	161	391
	% of dairy cow movements	15.1	78.3	70.3	47.2	69.2	35.0	12.5	60.8	32.8
Others	Cattle	191	31	3	20	75	72	6	39	437
	Dairy cow	158	30	3	10	55	63	0	25	344
	% of dairy cow	82.7	96.8	100.0	50.0	73.3	87.5	0	64.1	78.7
	Movements	31	4	1	3	6	7	3	10	65
	Dairy cow movements	23	3	1	2	4	4	0	6	43
	% of dairy cow movements	74.2	75.0	100.0	66.7	66.7	57.1	0	60.0	66.2

TABLE 7 Dairy cattle movements (frequency) and the number of cattle moved (animal head) between districts in northern Thailand with different regions as origin and destination from 2010 to 2017.

Regions	Origin		Destination	
	Movements	Cattle moved	Movements	Cattle moved
Central	2,629 (67.3%)	157,054 (87.1%)	2 (0%)	20 (0%)
Upper North	563 (14.4%)	1,652 (0.9%)	3,891 (99.6%)	180,135 (99.9%)
Lower North	31 (0.8%)	129 (0.1%)	6 (0.2%)	37 (0%)
Upper Northeast	0 (0%)	0 (0%)	1 (0%)	2 (0%)
Lower Northeast	440 (11.3%)	14,517 (8.1%)	2 (0%)	7 (0%)
East	196 (5.0%)	6,368 (3.5%)	0 (0%)	0 (0%)
West	47 (1.2%)	585 (0.3%)	4 (0.1%)	104 (0.1%)
Total	3,906 (100%)	180,305 (100%)	3,906 (100%)	180,305 (100%)

The influential districts identified for each stratified network

Given the two observations mentioned previously, influential districts were identified using the cutoff of network averages plus two standard deviations of each network metrics. This step was repeated for six networks to identify unique districts specific to each network. The comparisons of means between the network averages and the influential hot spot averages for each of the six networks are seen in Figure 2. Of the total 110 districts, 28 were determined to be influential based on their significant values on any of the centrality metrics (degree, eigen centrality, betweenness, and closeness). As seen in Figure 3, a total of 11 districts, including eight districts in the northern and one district in each of the central, eastern, and lower northeastern regions, were found to be significant on multiple metrics in at least one network. For example, Mueang Lamphun district from the Lamphun province in the northern region was found to be important for all five networks, except the within-north network. Specifically, it had a high degree in five networks and high eigen centrality in four networks. In addition, Ban Thi district in Lamphun province in the northern region was important for the full movement network, the slaughtering network, the growing and selling network, and the within-north network. This district showed particularly high betweenness in four networks, high closeness in two networks, and a high degree in one network. Moreover, Chai Prakan district in Chiang Mai province and Mae Suai district in Chiang Rai province were found to have frequent movement in both the growing and selling networks and the within-northern movement network. The geographic locations of these districts are seen in Figure 4 with their corresponding networks.

Discussion

The results from this study demonstrate the dynamic pattern of cattle movements in northern Thailand. From 2010 to 2016,

TABLE 8 Graph-level network metrics for full network and networks stratified for year, region, and primary purpose of dairy cattle movements between districts in northern Thailand from 2010 to 2017.

Networks		In degree	Out degree	Degree	Eigen centrality	Betweenness	Closeness	Reciprocity	Strength	Graph density	Transitivity	Average path length
Overall	2010–2017	21.18	17.85	10.54	0.99	0.1	0.3	0.01	70.38	0.32	0.19	3.07
By year	2010	9.71	9.92	4.79	0.97	0.1	1.3	0.01	43.79	0.59	0.28	2.28
	2011	7.1	4.53	3.5	0.96	0	1.21	0	24.98	0.28	0.21	1.27
	2012	7.69	6.14	3.8	0.98	0	1.09	0	23.08	0.25	0	1.36
	2013	7.23	6.37	3.54	0.97	0.02	1.03	0.01	23.78	0.34	0.21	1.96
	2014	7.27	6.48	3.6	0.99	0.01	0.95	0	19.37	0.28	0	1.4
	2015	7.67	5.73	3.83	0.99	0.02	0.69	0	17.22	0.25	0	2.11
	2016	10.38	8.25	5.14	0.99	0.05	0.97	0	24	0.5	0.15	1.85
	2017	4.21	3.98	2.05	1	0.07	0.21	0.01	22.35	0.22	0.16	2.93
By region	Central to North	41.57	47.23	23.41	0.99	0	1.18	0	125.19	1.53	0	1
	Other to North	7.64	2.74	3.81	0.99	0	1.22	0	18.94	0.12	0	1.52
	Within North	2.05	2.71	1.29	0.97	0.17	0.33	0.08	20.68	0.2	0.21	2.69
By purpose	Grow and sell	0.88	1.36	0.7	0.99	0.1	1.34	0.03	13.13	0.06	0.17	3.2
	Slaughter	53.34	45.85	26.38	0.98	0.02	1.21	0	152.14	1.86	0.12	2.12

the movement of dairy cattle together with the number of cattle moved had decreased. This could be influenced by the restriction of cattle movement during this period. The northern region of Thailand is an endemic area of several infectious diseases in cattle, such as FMD and bTB. FMD has been regularly reported in dairy and beef cattle in several provinces in northern Thailand. A previous study on FMD outbreaks from 2015 to 2017 reported an increasing number of FMD outbreaks, which peaked in 2016, while a significant reduction of FMD outbreaks was observed in 2017 (12). In addition, bTB was extensively investigated from 2011 to 2015 in northern Thailand by the DLD (11). The official reports on the detection of these infectious diseases resulted in many restrictions on cattle movement within and across the northern region of Thailand.

Overall, most cattle moved between districts for the purpose of slaughter, primarily male dairy calves. Male dairy calves are considered surplus animals in dairy cattle farming because they do not contribute to milk production. The central region is the most extensive dairy farming area in Thailand (13). Therefore, the exportation of male dairy calves from the central region to other regions of the country is expected. Moreover, male dairy calf is the main ingredient for roasted calf, which is a very popular dish in northern Thailand. Our results revealed that the consumption of male dairy calves in the region is very high, as indicated by the main cattle type imported from other regions of the country. The movements of male dairy calves would be expected to have a low risk of pathogen transmission since animals were shipped to abattoirs for slaughter. Although a significant proportion of these calves intended for slaughter were not shipped directly to abattoirs, many are moved to houses or farms. Information was not available to indicate how long these animals remained at the house or farm location prior to slaughter or whether they were in contact with other cattle in situations that could result in disease transmission. Without movements of male dairy calves to abattoirs, movements of dairy cows to houses or farms for the purpose of growing and selling were dominant. Most of these movements were within northern Thailand, which could be considered a risk for disease transmission in the region.

In 2010, most movements were for the purpose of slaughtering male dairy calves, but they were recorded as farm or house being the destination. This could be explained by two reasons: First, slaughtering male dairy calves was usually conducted at houses or farms at that particular period. The carcasses might be locally sold and consumed in the area close to where the slaughtering process was performed. Second, the truck drivers who were responsible for the registration of movement records at the origin of movements might be dealers or unaware of the address of the destination. In these cases, the address as shown in the national identification card of the truck driver is usually used as the destination of the movement. A more consistent and reliable record of dairy cattle movements should be emphasized as it can be

TABLE 9 District-level network metrics for the full network and networks stratified for the primary purpose of dairy cattle movement between districts in northern Thailand from 2010 to 2017.

Networks	Degree[a]	Eigen centrality[a]	Betweenness[a]	Closeness[a]	Average path length
Full	70.38 (4, 2368)	0.03 (0, 1)	52.93 (0, 1240.44)	0.33 (0.33, 0.48)	3.07
Growing, selling	13.13 (3, 162)	0.03 (0, 1)	50.67 (0, 1184.02)	0.34 (0.32, 1)	3.2
Slaughtering	152.14 (2, 2264)	0.06 (0, 1)	3.69 (0, 28)	0.42 (0.31, 1)	2.12

[a] Values of mean (median, maximum) are shown to describe the district-level network metrics.

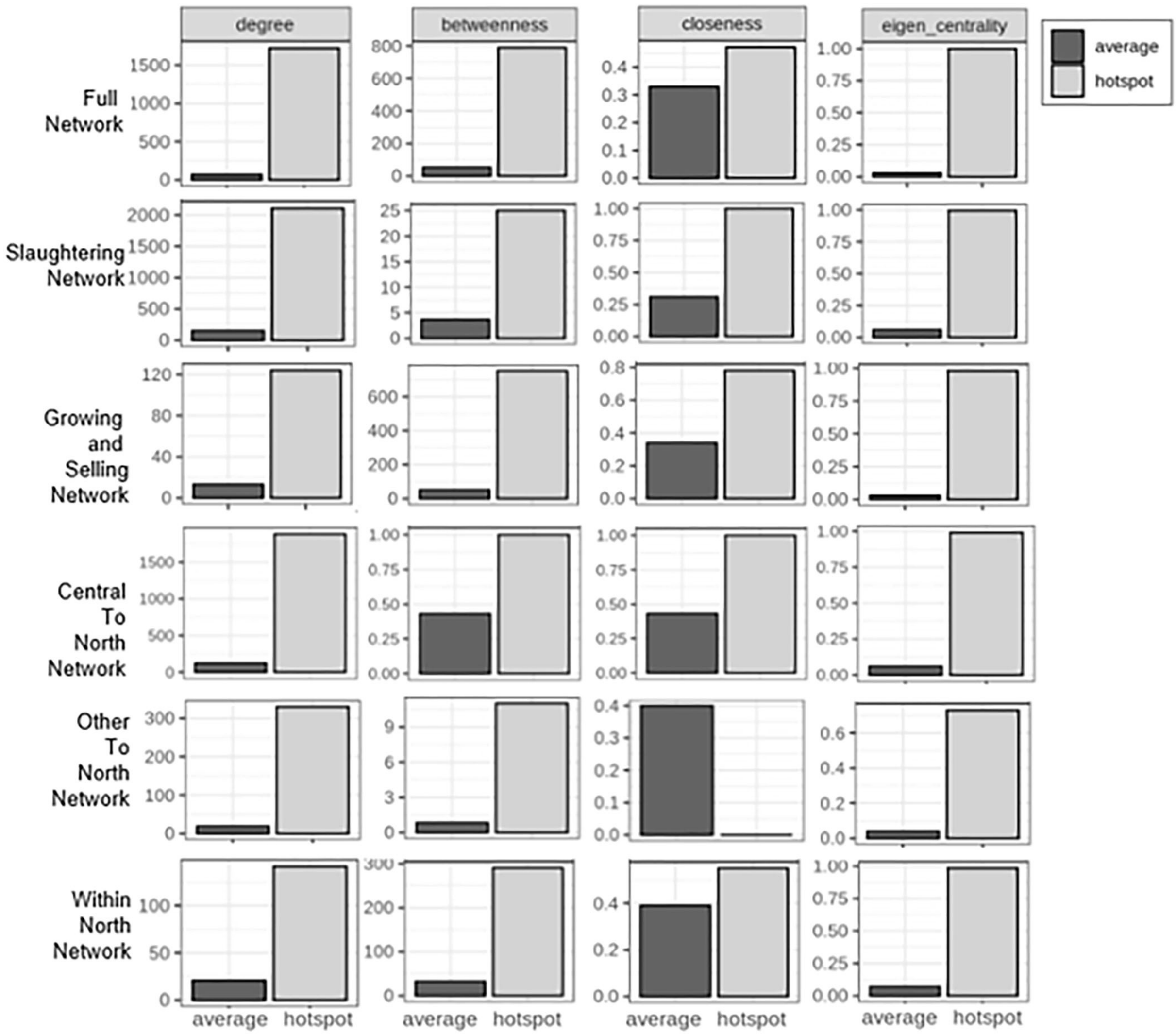

FIGURE 2
Comparisons of network averages and influential hot spot district averages. This bar plot shows the comparison of means between each of the six networks (full network, slaughtering network, growing and selling network, central-to-north movement, other-to-north movement, and within-north movement). The x-axis represents the groups in comparison: network average or influential hot spot average. The y-axis represents the value of means. This panel contains six rows showing the network evaluated and four columns showing the network metric calculated.

useful for disease investigation and outbreak controls in the region. Regarding this limitation, interpretation of the dairy cattle movement pattern, especially for the movements to destination premises rather than abattoir, should be cautiously made together with the movements for the purpose of growing and selling.

Social network analysis was conducted through quantification of metrics to investigate the hidden structures of

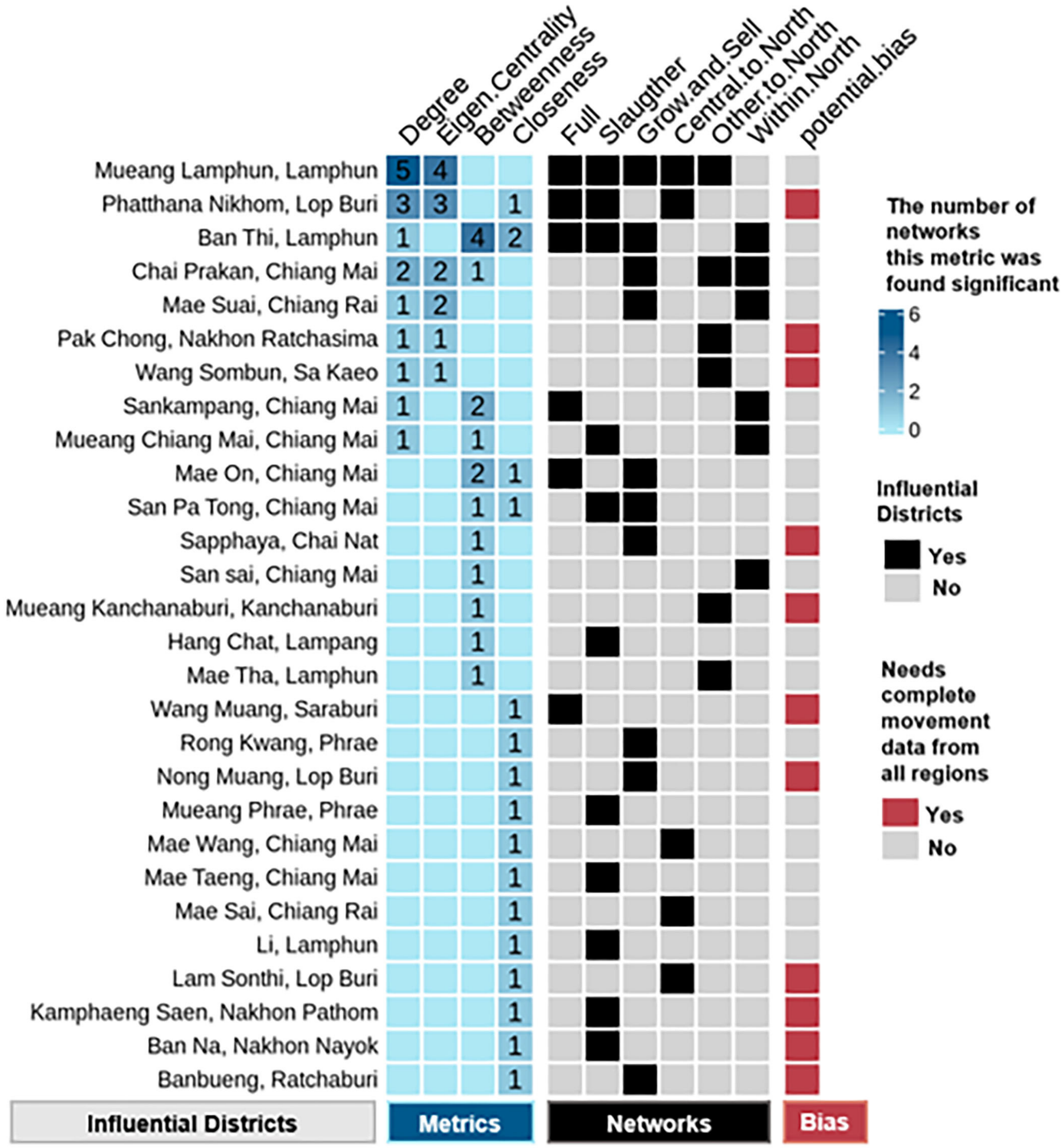

FIGURE 3
Influential districts are identified through social network analysis. The heatmap has three or four sections: districts, metrics, networks, and bias. The *district* section shows a complete list of districts identified as their network metrics exceeded the network mean plus two standard deviations of the metrics in corresponding networks. The *metric* section shows which of the four metrics were significant and how many networks were associated with these metrics. The *network* section shows which of the six networks the district had significant metrics. The bias section shows if the selected district was from the northern region, or complete movement data are needed to reevaluate its significance.

subnetworks by stratification and to identify districts with an increased risk of infection and spread of disease. These network metrics provided a fast and easy way to identify districts that were highly active and influential to other districts in the network. For example, Mueang Lamphun in Lamphun province showed a high degree and high eigen centrality in several networks, which could be the hub of receiving and distributing male dairy calves for fattening and slaughtering in the region. In addition, Ban Thi in Lamphun province showed the highest betweenness, which could be associated with its geographical

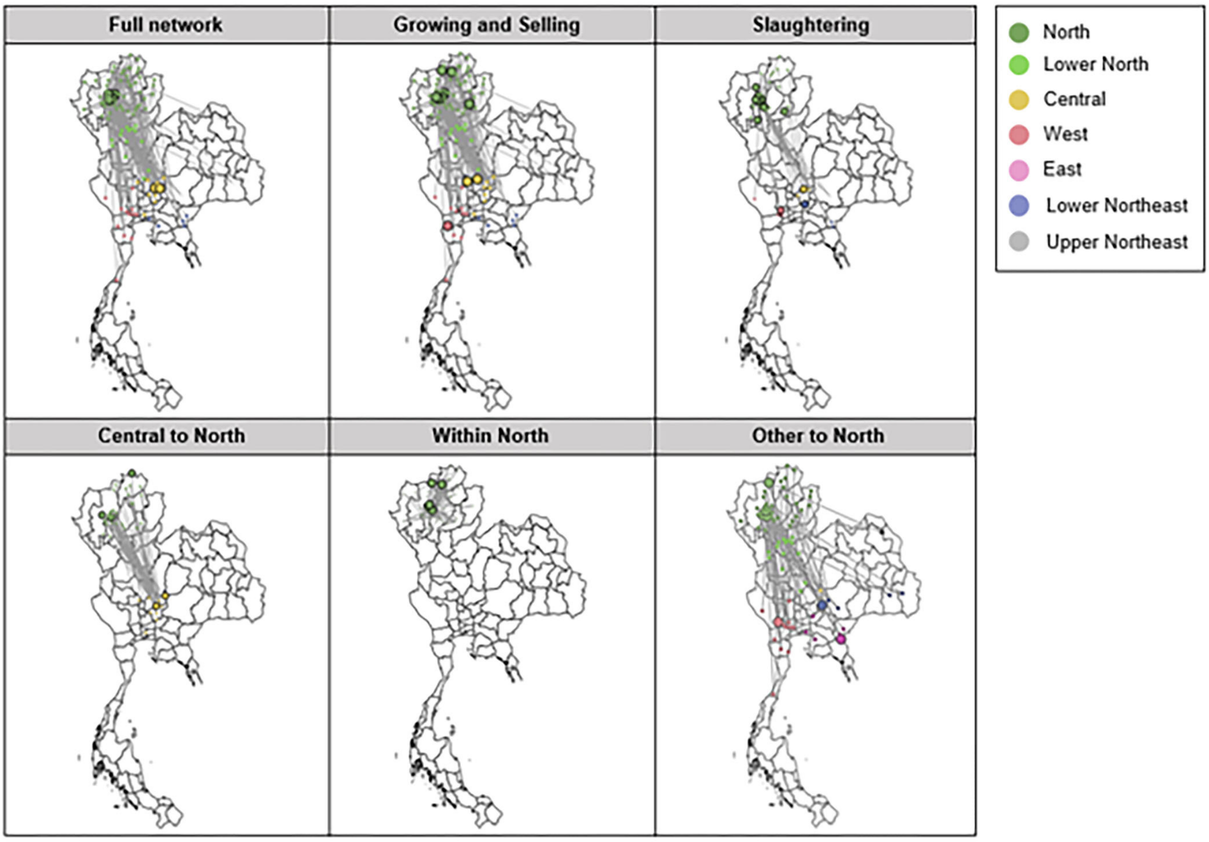

FIGURE 4
Full network, networks of movements with growing and selling as the primary purposes of movements, networks of movements from central to northern, within northern, and other to northern regions of Thailand, as overlaid on the geographical map of Thailand. Colored dots represent districts in the movement dataset. Big colored circles represent the influential districts of the dairy cattle movement for each network identified through social network analysis. Directed movement edges are simplified to a single edge between each pair of districts for visualization purposes.

location as it is in proximity with several dairy cattle crowded areas in Lamphun and Chiang Mai provinces. Several large dairy farms with >100 milking cows are located in the identified districts in central and other regions of the country (14), which were considered influential hot spots of origins of dairy cattle movements to the northern region of Thailand. Other influential districts located in northern Thailand are areas with a high density of smallholder dairy farms containing 20–100 milking cows (8). These districts are considered either hot spots of destinations as they mainly received dairy cattle for growing and selling or hot spots of origins of dairy cattle movements within the northern region of Thailand as they also distributed dairy cattle for growing and selling in other districts within the region. Moreover, our networks are north-centric; therefore, the influential districts identified from regions outside of the northern region need complete movement data from all regions to reevaluate their connectivity among these networks. Our approach of utilizing network metrics to identify influential districts can be easily applied once such data become available.

We found that the movement for slaughtering and the movement for growing and selling created distinctive networks with important implications for disease control and contact tracing. The network analysis identified potential targets to direct control efforts in more than 100 districts. Stratifying networks based on the purpose of movement helped realize the hidden pattern masked by a large number of slaughtering movements. More importantly, their differences in the network characteristics suggested that, during an epidemic, the spread of disease may be faster for the dense and localized slaughtering network than the growing and selling network. On the contrary, it may be difficult to implement disease control and contact tracing in the growing and selling network due to the large number of nodes involved. Further investigation may include incorporating geographical information because cattle producers need to travel across different provinces to move animals from one location to another. Incorporating road traffic and traveling routes could significantly improve the estimation of network structures. In addition, epidemic data can be simulated on the observed movement network to benchmark the performance of network metrics on recovering the chain of disease transmissions.

Even though several findings are reported in the current study, interpretation of the results should be cautiously made due to several limitations. One limitation is that only data on legal movements of dairy cattle were included for the analysis in the current study. Movements of beef cattle, buffaloes, and other non-ruminant animals can potentially contribute to the disease transmission in dairy cattle because most infectious diseases are not specific for only dairy cattle. Therefore, the implication of results from the current study for the transmission of diseases such as FMD, which can be transmitted through the movement of different domestic animals or through other mechanical vectors, can be very limited. Moreover, only records of movements between districts were available for analysis. Movements of dairy cattle within districts might more frequently occur, which can significantly contribute to the disease transmission between villages within each district. Information bias on types of premises of origins and destinations, and the primary purpose of the movement could occur in the current study because the records of these pieces of information could be subjectively and inconsistently made by the truck drivers. This bias can be minimized by applying a criterion to be used for recording these data at the movement registration, which can consequently improve the validity and accuracy of the data analysis.

Conclusion

Dairy cattle movements in the northern region of Thailand from 2010 to 2017 were analyzed. Decreasing trends in both dairy cattle movements and the number of cattle moved were observed from 2010 to 2016. In 2017, the movements of dairy cows for growing and selling increased from previous years. From the network analysis, several influential districts in northern and other regions were identified. These districts are key areas with potential for disease transmission among dairy cattle in the northern region of Thailand and the spreading of infectious diseases across regions in Thailand.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation. Requests to access these datasets should be directed to wang1927@umn.edu.

Author contributions

SB, SW, and KV designed the study. TS and AK collected, retrieved, and curated the data. YW analyzed the data. SB and YW wrote the manuscript. All authors read and approved the final manuscript.

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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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2022.961696/full#supplementary-material

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Stochastic partial budget analysis of strategies to reduce the prevalence of lung lesions in finishing pigs at slaughter

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Ante- and post-mortem inspections of food-producing animals at slaughter are mandatory activities carried out in many countries to ensure public health, animal health, and meat quality. In finishing pigs, lung lesions are the most frequent defects found in meat inspections. It is possible to implement managerial strategies on-farm to reduce the occurrence and spread of respiratory diseases, but such strategies come with additional costs that could impede implementation. This study assessed the economic impact of two strategies aimed at reducing the prevalence of lung lesions in finishing pigs at slaughter by improving the health conditions of the animals during the production cycle. First, a farrow-to-finish pig farm with 355 sows was modeled based on the current standard practice for finishing pig production in Sweden, using economic data, meat inspection data and biological variables from the literature and expert opinions. A partial budget analysis was then performed in which the baseline farm was compared with two hypothetical strategies aimed at reducing the occurrence and spread of respiratory diseases during pig production: (S1) avoiding mixing of litters after weaning and (S2) keeping purchased pregnant gilts separated from sows during gestation, farrowing and lactation. Both these strategies intended to reduce the occurrence of respiratory disease in finishing pigs at slaughter gave an average gain in annual net income (33,805 SEK in S1 and 173,160 SEK in S2, equal to 3,146€ and 16,113€, respectively, at the time of analysis), indicating that both were economically sustainable under the assumed conditions. The impact analysis of the two strategies revealed that the reduced prevalence of lung lesions when adopting one of the strategies was the most influential factor in net benefit change on the farm. Overall, the results suggest that with the increasing prevalence of lung lesions in Swedish pig production (as also observed worldwide in recent years), adopting an effective strategy to decrease respiratory infections will become more relevant and economically beneficial.

KEYWORDS

animal health, profitability, porcine, farrow-to-finish farms, meat inspection, pleurisy

Introduction

For decades, respiratory diseases have been the most important health problem in the global pig production industry (1). They also involve a large economic burden leading to losses for the producer, as pigs infected with respiratory pathogens are associated with an increased mortality rate (2), higher veterinary and medication costs, reduced average daily weight gain during the grower-finisher period (3, 4) and reduced feed conversion efficiency (5, 6). In addition, respiratory disease impairs pig welfare, since it affects health status and biological functioning, and thus causes distress in the animal (7).

In the European Union (EU), ante- and post-mortem inspections of food-producing animals are mandatory and encompass animal health surveillance, protection of public health and ensuring meat quality (8). Post-mortem meat inspection provides valuable indicators for monitoring the effect of disease control measures and estimating animal health status on-farm. Lung lesions are the most frequent defect found at meat inspection of slaughter pigs worldwide (9). Some of these lesions are indicative of specific pathogens, while others simply indicate a specific respiratory disease. Detection of pathological lung lesions during post-mortem inspection can lead to carcase devaluation and partial or whole carcase condemnation, which results in direct economic losses for primary producers. In addition, it can negatively affect slaughter operations by reducing slaughter line speed and increasing the labor required to handle the carcases, causing further economic losses.

The incidence of pleurisy and pneumonia at slaughter has increased globally in the past two decades (10) and the high prevalence of lung lesions and associated negative impacts on farm economics are a known issue (11). Since the prevalence of lung lesions in pigs depends on both infectious agents and environmental factors [e.g., air quality, production system, flooring type, number of pigs per pen and direct contact (nose-to-nose) between infected and susceptible pigs (11)], strategies to improve animal health, working environment and farm finances need to be implemented. Vaccination has been used to control pleurisy and lung lesions due to *Mycoplasma hyopneumoniae* (Mhyo) and *Actinobacillus pleuropneumoniae* (App). However, vaccination without proper hygiene management appears to be economically inefficient on farms with a high prevalence of App (12). In addition, appropriate gilt acclimatization strategies are crucial, since piglets tend to be infected with respiratory pathogens during farrowing or lactation (2, 11, 13). Regardless of the nature of the infection, it is possible to implement managerial strategies that reduce the occurrence and spread of respiratory diseases in finishing pigs. However, such strategies come with additional costs that may impede their implementation.

In this study, the costs of two such managerial strategies were assessed using partial budget analysis, a planning and decision-making tool that allows the evaluation of whether a new strategy in farm management or production practices will change the net benefit by considering the effects on net cost change and net benefit change (14–16). Stochastic partial budget analysis, where the range of values and the distribution of a selected indicator are considered, has become a popular tool for analyzing the economic effects of animal disease control (17, 18) and for decision-making to improve animal welfare (19). Measuring and estimating animal health and welfare in terms of economics is complex due to the multifactorial background and the limitations in the assumptions needed (20). However, economic research is important for the animal industry due to low-profit margins and for policymakers planning incentives to encourage animal welfare and health improvements.

The overall aim of this study was to assess the economic impact of two strategies aimed at reducing the prevalence of lung lesions detected at meat inspection of pigs by improving the health conditions of the animals during the production cycle. Two strategies that are currently recommended to Swedish pig producers to decrease the prevalence of lung lesions were considered: (i) avoiding mixing litters after weaning (S1) (21), and (ii) keeping purchased pregnant gilts separated from sows during gestation, farrowing and lactation (S2) (22). Stochastic partial budget analysis was used to measure how these two strategies affected the associated cost and net benefit change in finishing pig production.

Materials and methods

Baseline farm model

A farrow-to-finish farm with 355 sows (i.e., the average number recorded in 2018 and the reference year) was modeled using economic data from the WinPig® farm management software (23), and the meat inspection data were collected from Swedish abattoirs in 2018 and biological parameters were based on the literature. Additionally, personal communications from experts at the Farm and Animal Health Association (knowledgeable in pig production, disease control and economics) validated the baseline farm and supplied relevant information. Farrow-to-finish farms are integrated production systems that keep both nursery and finishing pigs and include all phases of the pig's life cycle, i.e., breeding, gestation, farrowing, lactation, weaning and growing the pig to a finishing weight of approximately 120 kg (24, 25). However, replacement sows are commonly recruited as 7-week pregnant gilts from the nucleus or multiplying herds. The total production period for adult sows lasts around 10 months: 4 months for breeding and gestation and six months for rearing the litter born to market weight. In the modeled farm, piglets were assumed to be crossbreeds between Landrace/Yorkshire bred with Duroc or Hampshire.

The baseline farm model was built in Microsoft Excel 2016 (Microsoft Corp., Redmond, WA, USA) and based on

TABLE 1 Summary of variables included in the baseline farm model. Costs shown are associated with the finishing phase of production on a yearly basis.

Variable	Mean value	Source
Number of finishing pigs sold	9,308	Meat inspection data 2018
Production days	97	WinPig 2018 (23)
Farm mortality (%)	1.8	WinPig 2018
Average production days for animals dead before slaughter	67.9	Expert opinion
Average daily feed intake (MJ NE)	26.4	WinPig 2018
Feed costs (SEK/MJ NE)	0.264	WinPig 2018
Working hours per pig per day	0.25	WinPig 2018
Labor costs (SEK/hour)	275	WinPig 2018
Cost of medicines, treatments and vet visits (SEK/pig)	5	WinPig 2018
Cost of carcass disposal (SEK/pig)	524	Svensk lantbrukstjänst, 2018 (26)
Prevalence of lung lesions at meat inspection (%)	19.2	Meat inspection data 2018
Average carcase weight for pigs without lung lesions (kg)	93.2	Meat inspection data 2018
Average carcase weight for pigs with lung lesions (kg)	92.3	Meat inspection data 2018
Meat sale price (SEK/kg)	18.58	KLS Ugglarps 2018 (27)
Deduction for lung lesions at meat inspection (SEK/pig)	−20	Personal communication
Loss of value due to carcase condition* (SEK/kg)	−0.30	KLS Ugglarps 2018

*It was assumed that carcases with lesions were allocated to a lower classification score (due to poorer general condition) and thus suffered a price penalty (28).

TABLE 2 Additional variables used in the partial budget analysis model for strategy S1 (avoiding mixing litters).

Variable	Mean value
Reduction in finishing pigs sold due to non-maximization of available space (%)	−2.5
Farm mortality (%)	0.9
Increased working hours at weaning stage (hours/pig)	0.0056
Decreased working hours at fattening stage (hours/pig)	0.17
Prevalence of lung lesions at meat inspection (%)	5.0

Source: personal communications from experts at the Farm and Animal Health Association.

current standard practices in finishing pig production in Sweden (Table 1). Since a farrow-to-finish production system was modeled, two sub-models were needed, one for the finishing phase and one for the gestation, farrowing, suckling and growth phases. Detailed figures about farrow-to-finish pig production are reported in Supplementary material 1.

The prevalence of lung lesions was estimated from data on pathological findings recorded at meat inspection of finishing pigs slaughtered in 2018 in the 10 largest abattoirs in Sweden. Upon meat inspection, up to five different lesions can be recorded at the carcase level using a standardized coding system (29). To match the baseline farm model, only farms that slaughtered between 7,000 and 12,000 pigs, which was the expected annual production of a farrow-to-finish farm with 355 sows, were considered. In total, 468,774 carcases from 53 different farms were represented in the data. Variables in the baseline farm model used in the partial budget analysis are listed in Table 1. The partial budget analysis was based on data for the finishing phase of production since this was assumed to have the greatest economic impact. Variables were assumed to have a normal distribution, with a 10% standard deviation (sd).

Strategies to reduce the prevalence of lung lesions

In scenario S1, it was assumed that the producer avoided mixing litters after weaning. In conventional integrated pig production, piglets are commonly mixed after weaning to ensure even groups of animals of approximately similar body weight. While this procedure allows the available space to be maximized, it also promotes the spread of infections, since pigs from different litters have differences in their immune system and bacterial flora. According to previous studies, avoiding mixing litters and rearing pigs in sibling groups seems to increase their performance, resulting in decreased detection of lung lesions at meat inspection (21). Table 2 summarizes the additional variables included in the partial budget analysis of model S1.

Most farms in Europe have quarantine units where purchased replacement gilts are held, either for a short period or until after farrowing or weaning (13). However, keeping the purchased groups of gilts from the existing sows for a longer time could increase their resistance to infections (30). The standard procedure in Sweden is to keep purchased groups of gilts in quarantine for 3 weeks and then place them in the same section as the existing sows once they have been inseminated (if not

TABLE 3 Additional variables from the baseline farm model used in the partial budget model for strategy S2 (keeping purchased gilts separated from existing sows).

Variables	Mean value
Farm mortality (%)	0.9
Decreased working hours at fattening stage (hours/pig)	0.17
Yearly cost of building a separate space for gilts (SEK)*	124,250
Prevalence of lung lesion at meat inspection (%)	3.0

Source: personal communications from experts at the Farm & Animal Health Association.
*Calculations are available in Supplementary material 2.

already pregnant at delivery). In scenario S2, it was assumed that purchased gilts were 7-weeks pregnant and were kept separated from the sows during gestation, farrowing and lactation. S2 was based on a Swedish study, where the results revealed that detection of pleurisy at slaughter decreased by 0.8–40.3% when keeping gilts separated from sows (22). Implementing S2 involves the establishment of a new facility to keep the gilts in quarantine for 9.5 weeks until the litter is weaned, an acclimatization strategy that has been proven to reduce the spread of bacteria from older parity sows to newly purchased gilts (30). The additional variables included in the partial budget analysis to model S2 are summarized in Table 3.

Strategies S1 and S2 both involve important practices that can control the spread of infections causing lung lesions (21, 22). Calculations on the space required for the quarantine of the purchased gilts in S2 and for keeping them separated from the other sows on the farm are provided in Supplementary material 2.

Stochastic partial budget analysis

Using partial budget analysis, a farm manager can evaluate whether a change in management or production practices will increase or decrease profit (14, 16). The method, however, does not determine profitability. It determines only the change in profitability, which is measured by the net benefit change as:

$$\text{Net benefit change} = \text{Total benefit change} - \text{Total cost change}$$

where, total benefit change includes costs saved and new revenues, while total cost change includes new costs and revenue foregone.

The stochastic partial budget analysis involves a budgeting approach using Monte Carlo simulations. To make the analysis stochastic, probabilities of occurrence need to be coupled to possible values of the key factors in a deterministic budget, thereby generating the probability distribution of possible budget outcomes (14, 19). The analysis in this study was performed using the Excel add-on @Risk (Palisade, Ithaca, NY) running 1,000 iterations.

In impact (sensitivity) analysis, several possible outcomes with a variety of input parameters are computed and subsequently displayed along with the probability to occur. This helps the decision-maker assess which risks to accept and which risks to avoid, allowing for optimal decision-making under uncertainty.

Partial budget model on the effects of strategy S1 (avoiding mixing litters)

In the scenario that involved avoiding mixing litters after weaning (S1), the total amount of finishing pigs on the farm was divided into four different categories, as listed in Table 4. The breakdown components of net benefit change in this case comprised costs saved, new costs and revenue forgone.

Costs saved

- Decreased costs of medicine, treatment and vet visits = cost per sick pig (SEK/slaughter pig) × number of sick pigs (with strategy S1 not adopted). It was assumed that the decrease in the number of sick pigs with the adoption of S1 was equal to half the change in the number of sick pigs B and all the changes in the number of pigs C, giving: $0.5\times(B(I) - B(II)) + (C(I) - C(II))$.
- Decreased labor hours at the finishing stage = decreased working hours × labor cost per hour.
- Decreased cost of daily feed for foregone pigs = average days fed before slaughter × average daily intake × feed cost × number of pigs that cannot be raised because of non-maximized use of space [i.e., D(II)].
- Decreased cost of carcass disposal = unitary cost for carcass disposal × decreased number of pigs dying of respiratory disease before slaughter: [C(I) - C(II)].
- Decreased deduction costs for lung lesions at meat inspection = unitary deduction price × change in the number of pigs showing lung lesions at meat inspection: [A(I) - A(II)].

Revenue foregone

Decreased carcase sales = average slaughter weight with strategy S1 (kg) × meat sales (price/kg) × increased number of finishing pigs sent to slaughter because of strategy S1 [D(II)].

New costs

- Increased feed costs = average daily intake × feed cost × average days fed × increased number of pigs without lesions at meat inspection: [C(I) - C(II)].
- Increased labor input, for pigs C with respiratory infections that die before slaughter = average work hours per day

TABLE 4 Categories of finishing pigs used in the partial budget model when either adopting or not adopting the strategy of avoiding mixing litters (S1).

Categories of pigs	If not adopting S1 (I)	If adopting S1 (II)
Number of pigs with no lung lesions at meat inspection (pigs A)	A(I)	A(II)
Number of pigs with lung lesions at meat inspection (pigs B)	B(I)	B(II)
Number of pigs with respiratory infections that die before slaughter (pigs C)	C(I)	C(II)
Number of foregone pigs* from not maximizing use of available space (pigs D)	D(I)	D(II)

*Foregone pigs = pigs that cannot be used in production because of sub-optimal use of space (i.e., uneven numbers of pigs in groups).

(at farm level) × labor cost × (average production days – average production days for animal dead before slaughter).

- Increased labor hours at weaning = increased working hours × labor cost.

In scenario S1 of avoiding mixing litters there were no increased benefits, so:

$$\text{Net changeS1} = \text{Costs saved} - \text{Revenue foregone} - \text{New costs.}$$

Partial budget model on the effects of strategy S2 (keeping purchased gilts separated)

In the scenario that involved keeping purchased gilts separated (S2), the total amount of finishing pigs on the farm was divided into three different categories, as listed in Table 5. The breakdown components for the cost and benefit analysis are displayed below.

New revenue

- Increased carcase sales = average slaughter weight with strategy S2 (kg) × meat sales (price/kg) × increased slaughter pig number because of strategy S2.

Costs saved

- Decreased costs of medicine, treatment and vet visits = unitary cost per sick pig (SEK/pig) × number of sick pigs (with strategy S2 not adopted). The decreased number of sick pigs was assumed to equal half the change in the number of sick pigs B and all the change in the number of pigs C, giving: 0.5×(*B(III)* - *B(IV)*) + (C(III) - C(IV)).
- Decreased labor hours at the finishing stage = decreased working hours × labor cost.
- Decreased cost of carcass disposal = unitary cost for carcass disposal × decreased number of pigs dying of respiratory diseases before slaughter: [C(III) – C(IV)].
- Decreased deduction costs for lung lesions at meat inspection = unitary deduction price × change in the number of pigs showing lung lesions at meat inspection.

New costs

- Increased feed cost = average increased daily intake × feed cost × average days fed × increased number of pigs without lung lesions at meat inspection: [C(III) - C(IV)].
- Increased labor input for pigs C, for decreased number of pigs with respiratory infections that die before slaughter = average work hours per day (at farm level) × labor cost × (average production days – average production days for animal dead before slaughter).
- Increased cost of a separate building for gilts.

In scenario S2 of keeping replacement gilts separated there were no foregone benefits, so:

$$\text{Net changeS2} = \text{New revenue} + \text{Costs saved} - \text{New costs.}$$

Results

Deterministic results of partial budget analysis are reported in Tables 6, 7, while stochastic results are given in Table 8.

Effects of avoiding mixing litters (strategy S1)

The deterministic results of partial budget analysis (Table 6) revealed the main factors influencing the net benefit change incurred by adopting S1. The left part of Table 6 shows the benefit change due to strategy S1 (including increased revenues and reduced costs). There was no increased revenue when adopting S1. In terms of reduced costs, the largest change was in decreased costs of daily feed for foregone pigs (157,317 SEK), followed by decreased labor costs in the finishing phase (59,826 SEK). The subtotal for benefit change when adopting strategy S1 was 291,936 SEK. Cost changes due to strategy S1, which are shown in the right part of Table 6, included decreased revenues

TABLE 5 Categories of finishing pigs used in the partial budget model when either adopting or not adopting the strategy of keeping purchases gilts separated (S2).

Categories of pigs	If not adopting S2 (III)	If adopting S2 (IV)
Number of pigs with no lung lesions at meat inspection (pigs A)	A(III)	A(IV)
Number of pigs with lung lesions at meat inspection (pigs B)	B(III)	B(IV)
Number of pigs with respiratory infections that die before slaughter (pigs C)	C(III)	C(IV)

TABLE 6 Deterministic effects on net benefit change of adopting strategy S1 (avoiding mixing litters).

Benefit change (SEK)		Cost change (SEK)	
New revenue		**Revenue foregone**	
	0	Decreased carcase sales due to sub-optimal use of space	196,295
Costs saved		**New cost**	
Decreased costs of medicine, treatment and vet visits.	3,693	Increased feed costs	58,050
Decreased labor hours at finishing stage	59,826	Increased labor input for pigs with respiratory infections that die before slaughter	2,001
Decreased cost of daily feed for foregone pigs	157,317	Increased labor hours at weaning	2,005
Decreased cost of carcass disposal	44,994		
Decreased deduction costs for lung lesions at meat inspection	26,106		
Subtotal	**291,936**	**Subtotal**	**258,351**
Net (benefit) change		**33,585 SEK**	

TABLE 7 Deterministic effects on net benefit change of adopting strategy S2 (keeping purchased gilts separated).

Revenue change (SEK)		Cost change (SEK)	
New revenue		**Revenue foregone**	
Increased carcase sales	210,716		0
Costs saved		**New cost**	
Decreased costs of medicine, treatment and vet visits	4,114	Increased feed costs	56,634
Decreased labor hours at finishing stage	67,753	Increased labor input for pigs	2,001
Decreased cost of carcass disposal	43,897	Cost of building for gilts	124,250
Decreased deduction costs for lung lesions at meat inspection	29,565		
Subtotal	**356,045**	**Subtotal**	**182,885**
Net (benefit) change		**173,160 SEK**	

and increased costs. Decreased revenues derived from decreased carcase sales (196,295 SEK). Increased feed cost was the largest contributor to increased costs (58,050 SEK). The subtotal cost change was 258,351 SEK. Deducting the subtotal cost change from the subtotal benefit change gave an annual net benefit change of 33,585 SEK (with sd of 44,569 SEK, a median of 35,392, and 90% central range: −44,828; 102,573. See Table 8) and a per-animal net benefit change of 3.61 SEK. At the time of the analysis (6 July 2022), the exchange rate between Swedish Krona and Euro was 1 SEK = 0.093€.

The results of the impact (sensitivity) analysis of strategy S1 are shown in Figure 1. The higher prevalence of lung lesions when mixing litters was the most influential factor in determining net benefit change, followed by the mortality rate if mixing litters (correlation coefficient 0.42 and 0.32, respectively). Feed cost was estimated to be positively correlated with net benefit change (0.20), and with a meat sale price for carcases with lung lesions (0.12). Meat sale price for carcases without lung lesions was strongly negatively correlated with net benefit change (−0.50), followed by foregone raised pigs (−0.49). Mortality and prevalence of lung lesions if not mixing litters were estimated to be negatively correlated with net benefit change (Figure 1).

Effects of keeping purchased gilts separated (strategy S2)

Results for the partial budget analysis related to changes under strategy S2 are provided in Table 7. Increased carcase sales

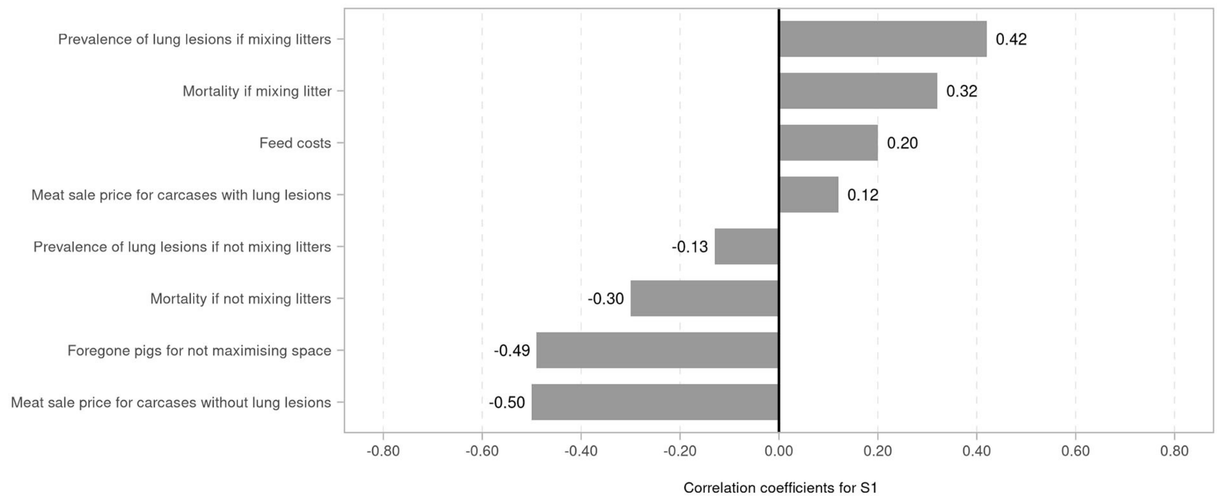

FIGURE 1
Tornado plot with correlation coefficients of drivers of net benefit change when avoiding mixing litters (strategy S1).

TABLE 8 Distribution of net benefit change of S1 and S2 from stochastic simulations.

Net benefit change	Median	sd	5%	95%
Net benefit change of S1	35,392	44,570	−44,823	102,573
Net benefit change of S2	171,124	382,189	111,627	237,716

gave 210,716 SEK, which was the most influential variable in determining benefit change, followed by decreased labor costs in the finishing stage and decreased cost of carcase disposal (67,753 SEK and 43,897 SEK, respectively). However, this increase in benefits was offset by increased costs of 182,885 SEK due to increased feed cost (56,634 SEK), labor cost (2,001 SEK), and cost of a new building for gilts (124,250 SEK). The annual net benefit change was 173,160 SEK (with sd of 38,218 SEK, a median of 171,124, and a 90% central range: 111,627; 237,716. See Table 8) and the per-animal increase in net benefit under S2 was 18.61 SEK.

A tornado diagram with correlation coefficients of drivers of net benefit change under S2 shows that the prevalence of lung lesions when not separating gilts was the most influential factor, with a correlation coefficient of 0.58, followed by meat sale price without lung lesions (correlation coefficient 0.44) (Figure 2). The mortality rate when not separating gilts was positively correlated with net benefit change (0.35). The cost of a building for gilts was most strongly negatively correlated with net benefit change (−0.36), followed by mortality rate if separating gilts (−0.35). The prevalence of lung lesions if separating gilts and feed costs was estimated to be negatively correlated with net benefit change (Figure 2).

Discussion

Using economic production data for an average Swedish farrow-to-finish pig farm with 355 sows, meat inspection data from 468,774 pig carcases derived from 53 different farms and expert opinions, this study explored the economic impact of adopting two strategies aimed at reducing the prevalence of lung lesions detected during meat inspections of finishing pigs. In both cases, the adoption of the strategy resulted in a positive change in net income.

Proper cleaning and vaccination programmes are performed more or less routinely on Swedish pig farms. The practices of mixing litters after weaning, to even out litter size, and keeping purchased gilts in quarantine for 3 weeks are also performed routinely. It is well-known that respiratory pathogens (e.g., Mhyo and App) are transmitted when pigs with differences in bacterial flora are placed next to each other or if there are too many pigs per unit floor area (30). However, the economic benefits of implementing strategies to control the spread of these respiratory pathogens have not been studied previously. This study assessed the costs associated with implementing two strategies [avoiding mixing litters after weaning and keeping the group intact until slaughter (S1) and keeping purchased pregnant gilts separated for a longer period (S2)], with both assumed to lead to a decreased spread of pathogens and thereby to more profitable pig production.

In both models, we adopted mean farm mortality of 1.80% and assumed it was normally distributed with 10% sd. The mean of farm mortality was derived from WinPig data for 2018. The WinPig dataset did not include the distribution and sd, so we consulted experts who had strong and long working experience on this perspective, and then we assumed

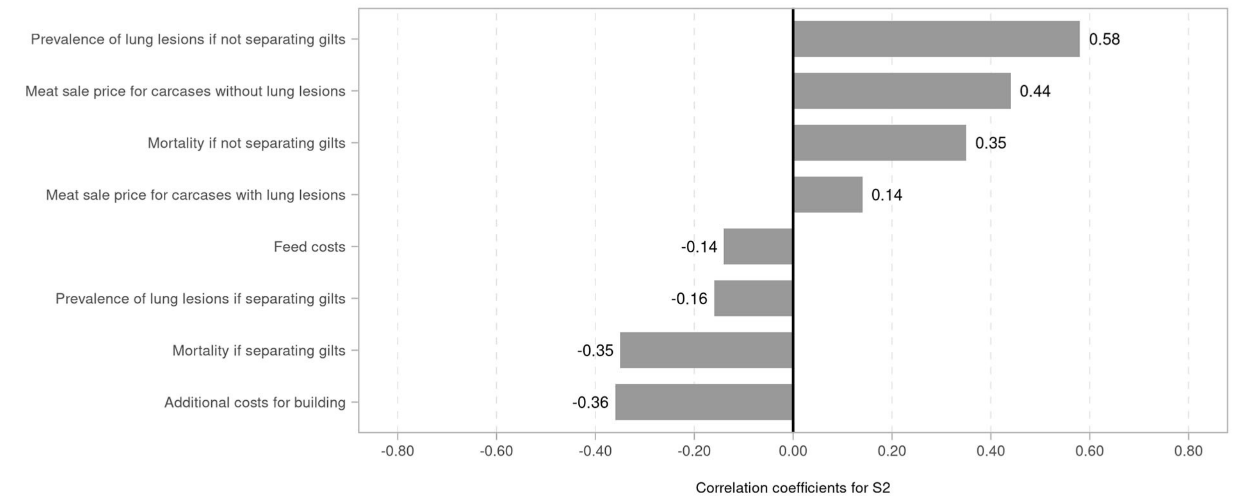

FIGURE 2
Tornado plot with correlation coefficients of drivers of net benefit change when keeping purchased gilts separated (strategy S2).

it had a normal distribution with sd of 10%. We also tried models with 5% and 15% sd, which showed that the results were comparatively robust; therefore, we showed and discussed only the results with 10% sd in the manuscript. Please see the sensitivity analysis for both strategies with 5% and 15% sd in the Supplementary material.

The strategy of avoiding mixing litters after weaning (S1) has the advantage of being easy and straightforward to implement; however, it comes with the risk of a negative revenue (Table 8) related to the number of pigs that cannot be used in production because of sub-optimal use of space. Farmers need therefore to evaluate risks and benefits according to their own situation. On the other hand, the strategy of separating newly purchased breeding stock (S2) was found to be associated with higher revenue but had the disadvantage of requiring a high initial investment in building the extended quarantine facility and a separate farrowing unit unless already existing buildings could be uses to elongate the adaptive phase for the replacers. If not, this high initial investment cost brings additional financial risks, increased costs per sow and increased amortization costs. This study did not consider future uncertainty or market volatility and assumed that the purchased gilts in S2 were pregnant at delivery.

The baseline farm model was based on an average farrow-to-finish pig farm, a system that requires high inputs of capital and labor but has great market potential and flexibility since the producer has control of the entire production cycle. Despite the suggested measures being meant to be applied during the earlier production phases (i.e., gestation and growing), the stochastic partial budget analysis was based on the finishing phase of production, which has the greatest impact on the entire production cycle and the most frequent detection of respiratory diseases. Raising healthy piglets is a key factor for a healthy and high-yielding pig population, so strategies S1 and S2 could also be applied on farrow-to-feeder farms. However, such producers would not be equally incentivised to adopt the strategies, as the greatest economic benefits (e.g., reduction of lung lesions detected at meat inspection, heavier and better carcases, and thus better meat sale price) would be gained by finishing pig producers buying healthier piglets who ultimately have less lung lesions and thus higher revenue for carcass sales.

The two proposed measures can have a positive impact on animal welfare as well, as healthier animals thrive better. The interest in explaining animal welfare in terms of production economics is increasing, e.g., Alvåsen et al. (26) performed an economic analysis on animal welfare for nurse sows, and Henningsen et al. (20) analyzed the empirical relationship between animal welfare and the economic performance of Danish pig farms, and Ahmed et al. (19) investigated the effect of space allowance on animal welfare and profitability for cattle fatteners. Those studies found various degrees of positive relationships between animal welfare indicators and economic outcomes, indicating the advantages of describing and estimating the economic effects of animal health and welfare. Although stochastic partial budget analysis can be applied in a variety of decision-making situations for farmers (31–37), there are some limitations to this method. First, it is restricted to evaluating only two alternatives and one of the alternatives is related to current operations. This meant that we compared cost-benefit change between the baseline farm and S1 and that between the baseline farm and S2, but the results cannot be used directly used to compare S1 and S2. Second, only cost and benefit changes that are affected by the intended decision are considered in the partial budget, and not the external situation.

If all areas affected by an intended change are not identified, the evaluation of the impacts of the change might be inaccurate. Third, the results obtained are estimates that are only as accurate as the original data used, and erroneous or inaccurate data can lead to biased results. Although stochastic partial budget analysis has been improved in this regard, caution is needed when using prior information for the analysis. In this context, it is a strength that this study used data from real studies (27, 37) as well as knowledge from experienced farm advisors. Even though the assumptions adopted in this study were likely to be reliable, there was a large variation in most input variables on commercial farms. This led to a large variation in the outcomes (Table 7), which requires extra caution when translating the results into operational activities. Additionally, the results of this study are mainly valid for the Swedish pig production system, which is known for having one of the highest animal welfare standards in the world (e.g., tail docking is banned, sows cannot be created during farrowing and suckling, and there is more space per pig in pens). Therefore, countries with lower animal welfare standards (e.g., where large groups of pigs are kept together, piglets are weaned at earlier age and sows are kept in crates during gestation) could be assumed to gain even higher economic benefits due to greater improvements in standards from adopting the two strategies analyzed in this study. If the study is replicated elsewhere, local production conditions must be taken into account.

There are a few additional limitations in this study that need to be addressed. First, there has been limited research on the economic benefits of implementing strategies to reduce the prevalence of respiratory diseases in pigs, which limited the input variables available for the analysis. This was also noted by Ahmed et al. (19), who experienced issues when estimating the exact health benefits in terms of production and profits of increased space allowance for cattle fatteners. Second, estimating the economic impact of strategies to reduce the prevalence of lung lesions is challenging, since co-infections of respiratory and non-respiratory (e.g., gastrointestinal) pathogens can occur simultaneously and it is impossible to separate their individual burden of disease. Third, if respiratory disease occurs during the growing phase or even earlier, there is a chance that any lung lesions will heal and not be detected during meat inspection (38). This means that the prevalence of lung lesions on-farm may be higher than recorded in meat inspections. Fourth, we did not explicitly consider feed conversion efficiency between pigs with and without lung lesions. However, we accounted for it indirectly in the model, by assuming that healthier pigs—because of S1 or S2—would be heavier at slaughter and have a better carcase score.

Potential economic benefits are the major incentives that could motivate producers to apply any preventive or suppressive measures. This study showed that by adopting the strategies of avoiding mixing litters and extending the quarantine period of purchased pregnant gilts, finishing pig producers can lower the prevalence of lung lesions recorded at slaughter and potentially increase the net economic benefit of production. However, Alvåsen et al. (39) point out the risk with assessing direct economic effects of animal health and welfare aspects, as it consists of both monetary and ethical values. The dilemma is that ethical values cannot be included in economic calculations and estimated in monetary terms. This also applied to the present study, where we identified a need for research covering ethical aspects of measuring animal welfare in economic terms. We also identified a need for further research on the economic aspects of animal health in pig production.

Conclusion

This study provided useful insights into the economic impact of two different strategies for reducing the prevalence of lung lesions in finishing pigs. Both strategies were found to be potentially economically sustainable under the assumed conditions. Impact analysis of the simulation models for the two strategies revealed that a higher prevalence of lung lesions if not adopting one of the strategies was the main factor determining the net benefit change on-farm. With the increasing prevalence of lung lesions (10), adopting an effective strategy to decrease respiratory infections will become more relevant and more economically beneficial. The results presented here can be used to support pig producers in choosing cost-effective management strategies to reduce the prevalence of lung lesions at slaughter by improving animal health and welfare.

Data availability statement

The raw data cannot be provided as it is covered by GDPR. All data required to replicate this work is provided (in aggregated form) in article tables and Supplementary material.

Author contributions

JJ: conceptualization, data acquisition, and writing—original draft, review and editing. WH: conceptualization, methodology and data analysis, and writing—review and editing. C-JE, IE, and AR: conceptualization, data acquisition, and review and editing. AC: conceptualization, data acquisition, writing—review and editing, funding acquisition, and project administration. All authors contributed to the article and approved the submitted version.

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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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2022.957975/full#supplementary-material

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The use of teat disinfectants and milking machine cleaning products in commercial Holstein-Friesian farms

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The aim of our study was to survey and analyze the use of pre- and post-milking teat disinfectants and milking machine cleaning products in large commercial Holstein-Friesian farms. A total of 43 Hungarian dairy farms with 31,430 cows with an average herd size of 731 cows were surveyed in 2014 by using a questionnaire *via* personal interviews. In the statistical analysis, we used ANOVA models and Tukey's multiple comparison method. Furthermore, seven in-depth individual interviews were conducted with farm managers. The results showed that 83.7% of the farms used different pre-milking disinfection methods (65.1% teat dips) and all of them applied post-milking disinfection. In the herds, chlorhexidine (42.9%) and other chlorine (21.4%) compounds were the most widely used active ingredients in the pre-milking disinfection, while iodine in the post-milking disinfection (53.8%). Lactic acid was ranked second in both disinfections (25.0 vs. 41.0%). In post-milking teat disinfection, the use of iodine and lactic acid combined with other active ingredients showed a significant relationship with SCC ($p = 0.0454$; $p = 0.0113$). In the milking machine cleaning process, the most frequently used active ingredients were sodium hypochlorite (80.0%) and sodium hydroxide (60.0%) as caustic detergents, while phosphoric acid (81.3%) as an acidic product. A significant relationship was found between the use of phosphoric acid combined with nitric acid, and the use of a combination of phosphoric acid, nitric acid, and organic acid and SCC ($p = 0.0483$; $p = 0.0477$). For farm decision-makers, the most decisive factor in the procurement of teat disinfectants was the active ingredient (3.4 on a scale of 1 to 10 where 1 was the most important), while regarding milking machine cleaning products the price (3.2).

KEYWORDS

dairy, udder health, mastitis, milk quality, teat disinfectants, milking machine cleaning

Introduction

Mastitis is one of the most frequent diseases of dairy cows and has well-recognized detrimental effects on animal wellbeing and dairy farm profitability (1). According to the National Mastitis Council (NMC) Recommended Mastitis Control Program, routine application of pre- and post-milking teat disinfectants during each milking is highly recommended to prevent new intramammary infections. Teat disinfectants should meet several requirements: (1) have proven germicidal efficacy, (2) prevent new intramammary infections, (3) maintain optimal teat condition and promote lesion healing, (4) not irritate the cow or the user, and (5) leave no residues in milk that could affect human health (2). Various teat cleaning disinfectants, including iodophor solution, iodine-based gel, sodium hypochlorite, dodecyl benzene sulfonic acid, chlorine, chlorhexidine, phenolic compounds, alcohol, and guava leaf extract, are used for pre-milking teat dipping (3). Iodine-based teat products are most used to disinfect teats before and after milking (4).

The milking machine cleaning has an important role in the reduction of bacterial numbers in milk (5). Cleaning and sanitation of milking equipment is a combination of chemical, thermal, and physical processes which need a minimum reaction time to be effective (6). The typical automatic cleaning process can be divided into three different main phases: pre-rinse, washing phase, and post-rinse. The pre-rinse phase is essential to remove most milk residues. During the washing phase, alkaline or acid detergents should be used. The alkaline detergent helps to remove organic deposits, such as milk protein and fat. Acid detergent is used periodically to remove mineral deposits from water and milk (7). A high proportion (67%) of liquid products used for cleaning and disinfection of Irish milking machines contain sodium hypochlorite, but some milk processors are now recommending the use of non-chlorine liquid detergent cleaning products such as sodium hydroxide or acids (5).

It is well-known that mastitis and milk quality are associated with teat disinfectants and milking machine cleaning products, but several practical aspects of their use, particularly regarding the milking machine cleaning practices, and their statistically confirmed impact on milk production in dairy cattle herds can contribute to the current knowledge in this field. Therefore, the aim of our study was to survey the practical use of pre- and post-milking teat disinfectants and milking machine cleaning products and analyze the associations between disinfection and cleaning practices, herd size, and milk production parameters in commercial Holstein-Friesian farms.

Materials and methods

Study design

The survey was drafted to define the use of teat disinfectants and milking machine cleaning products in commercial Holstein-Friesian farms and the views of farm managers on teat disinfection and cleaning practices. The drafted survey was reviewed by farm managers ($n = 2$), dairy cattle veterinary practitioners ($n = 3$), academic professionals ($n = 3$), and veterinary and animal science Ph.D. students ($n = 3$) to receive feedback on content. Based on collected feedback, revisions were made before the survey was sent to potential respondents. This study used a mixed-method approach, which combines the collection and analysis of quantitative and qualitative data. In the first part of this work, we collected data about the total number of cows, type of milking system and parlor, number of daily milkings, milk production parameters [lactation milk yield, somatic cell count (SCC), percentage share of marketed milk, and days in milk (DIM)], and we also surveyed the active ingredients of the used pre- and post-milking teat disinfectants and milking machine cleaning products (detergents and disinfectants), and their applications (e.g., disinfection and cleaning processes, the concentration of substances). The key factors in the procurement of teat disinfectants and milking machine cleaning products were also surveyed by evaluating their different characteristics on a scale of 1 to 10 ("1" for the most important, "10" for the least important factor). In the second part of the in-depth survey, structured individual interviews were conducted with dairy cattle farm managers. We used a questionnaire with open-ended questions that allowed the participants to convey their views on the aforementioned teat disinfection and milking machine cleaning practices.

Data collection

Commercial Holstein-Friesian farms were included in this survey based on the following criteria: use of computerized on-farm records, participation in milk recording, and willingness to provide data to the authors. A total of 43 Hungarian dairy farms were surveyed between September and October 2014 by using a questionnaire *via* personal interviews with farm managers ($n = 21$; 48.8%), veterinarians ($n = 14$; 32.6%), shift supervisors ($n = 5$; 11.6%), or division heads ($n = 3$; 7.0%), who had access to farm records and were familiar with the milking procedures in the studied dairy units. Furthermore, we had in-depth, structured individual interviews with seven farm managers (out of the 21). The participants took part in the survey voluntarily and remained anonymous. Each participant was required to sign a written consent before they began the survey. Each questionnaire has been coded to detect inaccuracies in data entry. The obtained data were processed in MS Excel (Microsoft Corporation, Redmond, WA, USA).

A total of 31,430 cows were kept on the 43 farms, which corresponded to 17.8% of the 176,753 Hungarian dairy cow population on 458 performance-tested farms according to the official statistical data during the time of the survey (8, 9). The smallest surveyed farm had 56 cows, whereas the largest had 2,500; the average herd size was 731 ± 508 (milking + dry cows). All of the seven regions of Hungary were covered in the survey (min. 3 and max. 14 dairy farms per region were involved). The seven, individually interviewed farm managers represented a total of 6,130 cows with an average herd size of 876 ± 779 ($n = 7$; min. 300; max. 2500).

Total annual milk production per farm was 6,712,655 kg ($n = 43$; min. 321,484 kg; max. 22,522,000 kg), of which 96.8% was marketed (min. 90.0%, max. 99.3%). The average lactation milk yield was 9,716 kg ($n = 41$; min. 5,409 kg; max. 11,915 kg), average milk fat content was 3.7% ($n = 41$; min. 2.97%; max. 4.16%), average milk protein content was 3.3% ($n = 41$; min. 3.17%; max. 4.2%), and average SCC was 419,000 ($n = 38$; min. 188,000; max. 936,000), respectively. The average length of lactation was 373 days ($n = 26$; min. 310 days; max. 545 days).

More than half of the milking parlors had a herringbone design (57.8%), followed by parallel (20.0%), rotary (17.8%), and polygon (4.4%) milking systems. The average age of the milking systems was 11.7 years ($n = 41$; min. 1 year; max. 28 years). The cows were mostly milked twice a day (62.8%), but 41.9% of the farms milked the cows three times and 4.7% four times a day. On four farms different cow groups were milked differently (usually cows were milked more frequently until 30 DIM). The vast majority of the farms (85.4%) used traditional elastic teat liners and 14.6% silicone ones. The teat cups were disconnected automatically on all farms, except for the smallest one.

All herds ($n = 43$) were free from tuberculosis, brucellosis, and bovine leukosis, but 34.9% of the farms were also free from either IBR (25.6%), BVD (4.7%) or the five diseases (4.7%). The diseased cows (e.g., clinical mastitis cases) were kept in separate hospital barns on 59.5% of the surveyed farms ($n = 42$). In the other herds, they were isolated within the maternity barn.

Statistical analysis

The surveyed farms represented all farm sizes, milking systems, milking parlor types, and geographical regions in Hungary. Teat disinfection and milking machine cleaning practice outcome measures were analyzed with ANOVA models. All models included the herd size (1–400, 401–800, and >800 cows), milking parlor type (herringbone, parallel, rotary, polygon), and number of daily milkings (two times, more than two times) as explanatory variables. Consequently, bias caused by data imbalance related to these variables was eliminated from the resulting estimates. For each pre- and post-milking teat disinfection and milking machine cleaning practice outcome, the basic model included only the three main management variables listed above. Next, each management explanatory variable was added to the basic model one by one separately (Tables 1, 2). The normality of the residuals (the difference between the raw data and the model prediction) is checked by quantile comparison plots (QQ-plot), and no deviation from normality was found. Differences between the means of the outcome variables in the layers of the explanatory variables were evaluated by Tukey's multiple comparison method applying the R package multcomp. Statistical analyses were performed in R version 4.1.2 (10). The level of significance was set to 0.05.

TABLE 1 The analyzed teat disinfection explanatory variables.

Variable	
Use of pre-milking disinfectant	Yes
	No
Component number of a pre-milking teat disinfectant	One
	Two
	Use of impregnated paper
Active ingredient of pre-milking teat disinfectant	Alcohol
	Chlorine
	Chlorine/alcohol
	Chlorine/iodine
	Chlorine/lactic acid
	Lactic acid
	Other acid
Using chlorine as a pre-milking teat disinfectant	Yes
	No
Using lactic acid as a pre-milking teat disinfectant	Yes
	No
Using alcohol as a pre-milking teat disinfectant	Yes
	No
Component number of a post-milking teat disinfectant	One
	Two
Active ingredient of a post-milking disinfectant	Chlorine
	Iodine
	Iodine/chlorine
	Iodine/lactic acid
	Iodine/lactic acid/chlorine
	Iodine/other
	Lactic acid
	Lactic acid/chlorine
	Lactic acid/other
	Other
Use of iodine as a post-milking disinfectant	Yes
	No
Use of lactic acid as a post-milking disinfectant	Yes
	No

To support confirmatory analysis with newly collected data, in cases of marginally significant differences (i.e., when the p-values were >0.05 and <0.1), we carried out power analysis assuming equal sample sizes in the groups compared. The calculations were carried out applying the method of Cohen (11), implemented in the package pwr of R version 4.1.2 (10). The effect sizes (i.e., the ratios of mean differences to residual standard deviations) were set to 0.88, which is the smallest effect size obtained from the ANOVA models in this paper. The required power was set to 0.8, and the family-wise error rate related to the multiple comparisons of group means was set to

TABLE 2 The analyzed milking machine cleaning explanatory variables.

Variable	
Phases of cleaning	3
	5
	Both
Rinsing	After cleaning
	Before cleaning
Acid concentration	1%
	Not 1%
Caustic concentration	1%
	Not 1%
Disinfectant concentration	≤1%
	>1%
Daily cleaning	After every milking
	Not after every milking
Number of weekly acid cleanings	1–3.5
	7–21
Number of yearly manual cleanings	0–12
	24–52
Caustic ingredients	Sodium hydroxide
	Sodium hydroxide/sodium hypochlorite
	Sodium hypochlorite
	Other
Acid ingredients	Nitric acid
	Organic acids
	Phosphoric acid
	Phosphoric acid/nitric acid
	Phosphoric acid/nitric acid/organic acid
	Phosphoric acid/sulfuric acid
Use of phosphoric acid	Yes
	No
Use of nitric acid	Yes
	No
Use of sulphuric acid	Yes
	No
Use of disinfectants	Yes
	No
Disinfectant ingredients	Hydrogen peroxide
	Peracetic acid
	Peracetic acid/hydrogen peroxide

0.05. We applied Bonferroni corrections to adjust p-values in multiple comparisons. In summary, the power analysis revealed that the smallest eligible sample size for the new balanced study was 23.

Results

There was no significant relationship between the herd size and the milking parlor type, and the lactation milk yield and SCC ($p \geq 0.108$ and $p \geq 0.192$). The percentage share of marketed milk tended to be higher in parallel parlors vs. herringbone ($p = 0.0772$) and DIM tended to be higher in herringbone parlors vs. polygon ($p = 0.0729$), and in herds with >800 cows vs. 1–400 cows ($p = 0.0892$) by Tukey's multiple comparison method. A significant relationship was found between the number of daily milkings and lactation milk yield ($p = 0.0182$).

Pre-milking teat disinfectants

Vast majority of the surveyed farms (83.7%) used different pre-milking teat disinfection procedures, 65.1% used disinfectant teat dips, 14.0% disinfectant wash or foaming, and 4.7% impregnated papers (Supplementary Table 1). No significant association was found between the use of pre-milking disinfection and the studied milk production parameters (lactation milk yield, percentage share of marketed milk, DIM, SCC; $p \geq 0.3356$). Six out of seven (85.7%) personally interviewed farm managers said that pre-milking teat disinfection should always be applied and four out of seven (57.1%) considered disinfectant dip or foaming disinfectant wash to be the ideal method.

Out of the farms applying pre-milking teat disinfection 72.2% used one-component pre-milking disinfectants exclusively, 8.3% both one- and two-component disinfectants, 13.9% only two-component products, while two large dairies (5.6%) used disinfectant impregnated paper (Supplementary Table 2). The use of one component pre-milking disinfectant and the use of impregnated paper showed significant association with DIM ($p = 0.0125$; $p = 0.0376$) and the use of two-component pre-milking disinfectant tended to associate with SCC and DIM ($p = 0.0865$; $p = 0.0716$). All seven personally interviewed farm managers preferred one-component pre-milking disinfectants, because of their practical use primarily (71.4%), that is, they exclude the possibility of human errors, but the longer shelf life and sales discounts were also mentioned (14.3% each).

The most used active ingredient of the pre-milking disinfectants was chlorhexidine (42.9%), followed by lactic acid (25.0%) and other compounds of chlorine (chlorine dioxide and triclosan together: 21.4%). The pre-milking disinfectants containing compounds of chlorine were used in almost two-thirds (64.3%) of the commercial dairy units, while disinfectants with iodine were the least preferred products (7.1%) (Figure 1). No significant association was found between the active ingredients and the studied milk production parameters ($p \geq 0.1535$) (Tables 3–6). Several farms used multiple pre-milking teat disinfectants at the same time.

Three out of the seven interviewed farm managers (42.9%) considered chlorhexidine to be the optimal active ingredient in pre-milking disinfection, although another three managers (42.9%) considered iodine to be equally good. One manager

(14.3%) also mentioned lactic acid as an optimal active ingredient. Six out of seven farm managers (85.7%) named efficacy as the major reason for choosing a certain active ingredient for pre-milking disinfection.

Post-milking teat disinfectants

All the surveyed farms applied post-milking teat disinfection and 67.5% of the farms exclusively used one-component post-milking disinfectants, 27.5% two-component products alone, and a further 5.0% both (Supplementary Table 3). No significant relationship was found between the component number of the post-milking disinfectant and the studied milk production parameters ($p \geq 0.1300$). Six out of seven interviewed farm managers (85.7%) preferred one-component post-milking disinfectants, because of their practical use primarily (57.1%), but the efficacy, sales discount, and longer shelf life were also mentioned (14.3% each).

Iodine was the most used active ingredient in post-milking disinfection on the farms (53.8%), followed by lactic acid (41.0%) and various compounds of chlorine (chlorhexidine, chlorine dioxide and sodium chlorite) with a total share of 36.0% (Figure 2). The use of iodine and lactic acid combined with other active ingredients showed a significant relationship with SCC ($p = 0.0454$; $p = 0.0113$) (Tables 3–6). Several farms used multiple post-milking teat disinfectants at the same time. Six out of seven individually interviewed farm managers (85.7%) considered iodine as the optimal active ingredient in post-milking disinfection, although two managers (28.6%) also named lactic acid and chlorhexidine. The major reason for the choice of an active ingredient was the efficacy (100%), but one farm manager (14.3%) underlined the effectiveness against *Staphylococcus aureus* and another one (14.3%) mentioned the importance of skin integrity, referring to the fact that iodine dries the skin.

The key factors in the procurement of teat disinfectants were also surveyed, the farm decision-makers evaluated the different characteristics of pre- and post-milking disinfectants on a scale of 1 to 10 ("1" for the most important, "10" for the least important factor). Figure 3 shows that the active ingredient was the most decisive procurement factor for a teat disinfectant, followed by price and ease of use. The advertisement was considered as the least important factor in the procurement, and sales discounts were not that important either.

The seven personal interviews confirmed the results of the survey, since the most important teat disinfectant characteristic for all seven farm managers was efficacy, followed by color of the product (85.7%), good value/price ratio (71.4%), reliable supply (57.1%), no skin drying effect (42.9%), and proper teat skin coverage (28.6%). The solution color makes it easy for the farm managers to check if the disinfectant was applied correctly.

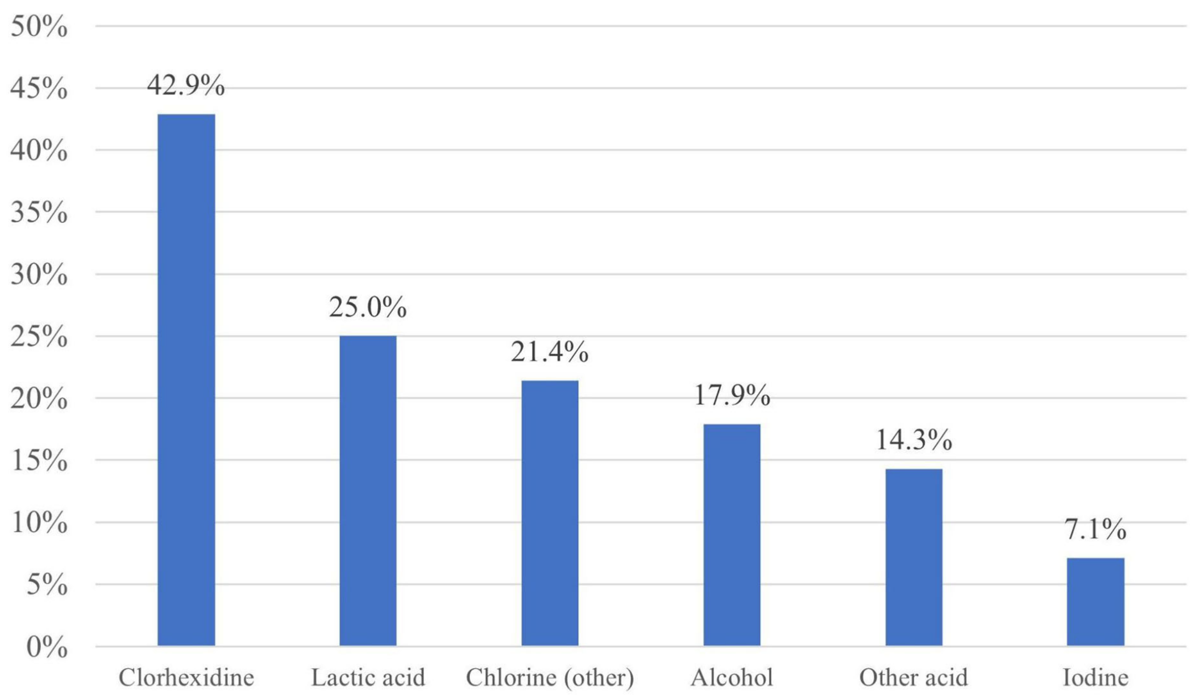

FIGURE 1
Active ingredients of the pre-milking teat disinfectants ($n = 28$).

TABLE 3 Relationships between somatic cell count (SCC/ml) and pre- and post-milking disinfectants.

Variables	Layers	Mean	SD	*n*	*P*-value
Active ingredient pre-milking disinfectant	Alcohol	472.333	111.114	3	
	Chlorine	481.000	190.711	12	0.8680
	Chlorine/alcohol	386.000		1	0.9295
	Chlorine/iodine	365.000		1	0.4908
	Chlorine/lactic acid	390.000		1	0.5497
	Lactic acid	459.800	217.520	5	0.6347
	Other acids	220.000		1	0.3745
Using chlorine as a pre-milking disinfectant	No	437.333	182.863	9	
	Yes	460.867	174.184	15	0.5010
Using lactic acid as a pre-milking disinfectant	No	453.333	171.810	18	
	Yes	448.167	196.695	6	0.6604
Using alcohol as a pre-milking disinfectant	No	452.300	187.096	20	
	Yes	450.750	100.470	4	0.6088
Active ingredient of a post-milking disinfectant	Chlorine	424.200	101.726	5	
	Iodine	408.889	183.767	9	0.0454*
	Iodine/chlorine	582.500	307.592	2	0.3956
	Iodine/lactic acid	566.667	202.073	3	0.1801
	Iodine/lactic acid/chlorine	395.000		1	0.6219
	Iodine/other	550.000		1	0.7513
	Lactic acid	429.500	183.903	8	0.9436
	Lactic acid/chlorine	250.000	42.426	2	0.1358
	Lactic acid/other	643.000	414.365	2	0.0113*
	Other	350.000		1	0.2030
Using iodine as a post-milking disinfectant	No	444.333	184.796	18	
	Yes	479.722	183.276	18	0.1841
Using lactic acid as a post-milking disinfectant	No	491.095	201.472	21	
	Yes	421.333	148.430	15	0.2671

*Significant relationships ($p < 0.05$).

Two farm managers (28.6%) underlined the importance of skin coverage and the ability of the product to stay on the skin, that is, the disinfectant should not drip off, it should stay on the teat for a long enough time so that it can take full effect. Other factors, namely, number of milking sessions, package size, easy to use, small per-unit cost, viscosity and distributor's recommendation were mentioned once by the farm managers (14.3% each).

Milking machine cleaning products (detergents and disinfectants)

About 59.5% of the surveyed farms used a 5-phase cleaning system (pre-rinse; caustic wash; rinse; disinfection; final rinse), 35.7% applied three-phase systems (pre-rinse; sanitizing wash including cleaning and disinfection; final rinse), and 4.8% used both systems depending on timing and duration of the milking session (Supplementary Table 4). We found no significant relationship between the type of cleaning and the studied milk production parameters ($p \geq 0.1522$). Four out of seven interviewed farm managers (57.1%) considered the three-phase cleaning systems better than the 5-phase ones. 74.4% of the farms performed the final rinsing cycle immediately after disinfection and 25.6% directly before the next milking session ($n = 39$). The time of the final rising tended to associate with SCC ($p = 0.0634$). All the interviewed farm managers considered rinsing to be optimal directly after disinfection.

Milking machines were cleaned twice a day in 73.8% of the farms, three times a day in 21.4%, but only once a day in 4.8%, respectively (Supplementary Table 5). It can be stated that the milking machinery was not cleaned after every milking on many farms (especially in the case of three milking sessions a day), despite the fact, that all seven interviewed farm managers said that cleaning should be performed after each milking optimally. There was no significant relationship between daily cleaning and the studied milk production parameters ($p \geq 0.1254$).

The number of acid descaling washes per week revealed a very diverse picture: it ranged from once a week (in 11.9%

TABLE 4 Relationships between lactation milk yield (kg) and pre- and post-milking disinfectants.

Variables	Layers	Mean	SD	*n*	*P*-value
Active ingredient pre-milking disinfectant	Alcohol	10.284	415	4	
	Chlorine	9.551	1.056	11	0.1535
	Chlorine/alcohol	9.853		1	0.1896
	Chlorine/iodine	10.200		1	0.3134
	Chlorine/lactic acid	10.300		1	0.3558
	Lactic acid	9.059	921	5	0.4425
	Other acids	8.483	81	2	0.3158
Using chlorine as a pre-milking disinfectant	No	9.400	964	11	
	Yes	9.833	1.056	16	0.3409
Using lactic acid as a pre-milking disinfectant	No	9.757	1.060	20	
	Yes	9.371	925	7	0.9721
Using alcohol as a pre-milking disinfectant	No	9.512	1.108	21	
	Yes	10.165	374	6	0.4245
Active ingredient of a post-milking disinfectant	Chlorine	8.737	1.821	5	
	Iodine	9.974	1.075	11	0.9038
	Iodine/chlorine	8.900	1.838	2	0.5961
	Iodine/lactic acid	8.020	2.321	3	0.8750
	Iodine/lactic acid/chlorine	10.200		1	0.9197
	Iodine/other	8.400		1	0.2156
	Lactic acid	9.112	1.316	8	0.5454
	Lactic acid/chlorine	9.210	948	2	0.8105
	Lactic acid/other	9.356	786	2	0.9439
	Other	10.600		1	0.2637
Using iodine as a post-milking disinfectant	No	8.945	1.369	18	
	Yes	9.603	1.308	21	0.7381
Using lactic acid as a post-milking disinfectant	No	9.358	1.435	23	
	Yes	9.215	1.285	16	0.4736

of the farms) to 21 times a week (11.9%), that is, after every milking, but in most cases, it was performed daily (26.2%) or 2 (23.8%) to 3 (16.7%) times per week (Supplementary Table 6). There was no significant relationship between the number of weekly acid cleaning and the studied milk production parameters ($p \geq 0.2588$). Even the opinions of the interviewed farm managers on the optimal number of acid descaling washes per week differed: three out of seven (42.9%) said an acid wash should be performed after every milking, another three (42.9%) were in favor of an acid wash after every fourth caustic wash, and one manager (14.3%) said that one acid wash a day would be optimal.

There was an even greater deviation regarding the average yearly number of occasions when the milking machine was disassembled so that its parts could be cleaned thoroughly, manually. In 29.4% of the farms, this took place every week (52 × a year) and in 17.6% every month (12 × a year). At the same time, 11.8% of the farms never cleaned the milking machines manually (Supplementary Table 7). There was no significant relationship between the number of manual machine cleaning per year and the studied milk production parameters ($p \geq 0.1113$). The opinions of the interviewed farm managers on the optimal number of manual cleaning sessions per year also greatly varied. Most commonly (28.6% each) a manual cleaning in every 6 months (that is, 2 × a year) and every 3 months (that is, 4 × a year) was considered as optimal, but one manager (14.3%) said that the weekly (52×) cleaning would be optimal.

Sodium hypochlorite was the most preferred active ingredient of caustic detergents (80.0% of the farms used it), followed by sodium hydroxide (60.0%) and potassium hydroxide (20.0%) (Figure 4). There was no significant relationship between the used caustic detergent and the studied milk production parameters ($p \geq 0.426$). Three out of seven personally interviewed farm managers (42.9%) were not aware of the active ingredient or the name of the caustic detergent they used on their farm; one (14.3%) could name the product, another (14.3%) could name the distributor, and two of them (28.6%) knew that they used a detergent containing chlorine.

TABLE 5 Relationships between the share of marketed milk (%) and pre- and post-milking disinfectants.

Variables	Layers	Mean	SD	*n*	*P*-value
Active ingredient pre-milking disinfectant	Alcohol	96.3	1.8	4	
	Chlorine	96.8	1.8	11	0.641
	Chlorine/alcohol	93.5		1	0.301
	Chlorine/iodine	98.8		1	0.260
	Chlorine/lactic acid	ND		0	
	Lactic acid	97.7	1.3	5	0.407
	Other acids	97.4	1.2	2	0.761
Using chlorine as a pre-milking disinfectant	No	97.1	1.5	11	
	Yes	96.8	1.9	15	0.733
Using lactic acid as a pre-milking disinfectant	No	96.6	1.8	20	
	Yes	97.9	1.3	6	0.184
Using alcohol as a pre-milking disinfectant	No	97.1	1.6	20	
	Yes	96.3	2.2	6	0.444
Active ingredient of a post-milking disinfectant	Chlorine	96.4	2.0	4	
	Iodine	97.1	1.5	13	0.675
	Iodine/chlorine	96.0	3.8	2	0.553
	Iodine/lactic acid	95.9	5.1	3	0.743
	Iodine/lactic acid/chlorine	98.0		1	0.970
	Iodine/other	ND		0	0.313
	Lactic acid	97.1	1.1	6	0.940
	Lactic acid/chlorine	98.1	0.1	2	0.341
	Lactic acid/other	96.9		1	0.657
	Other	96.5		1	0.889
Using iodine as a post-milking disinfectant	No	96.8	2.1	17	
	Yes	96.7	2.4	17	0.880
Using lactic acid as a post-milking disinfectant	No	96.3	2.8	18	
	Yes	97.2	1.6	16	0.403

ND, no data.

Phosphoric acid was the most used acid (in 81.3% of the farms), followed by nitric acid (in 28.3%), sulfuric acid (in 21.9%), and organic (acetic and citric) acids (in 12.5%) (Figure 5). A significant relationship was found between the use of phosphoric acid combined with nitric acid and the use of a combination of phosphoric acid, nitric acid, and organic acid and SCC ($p = 0.0483$; $p = 0.0477$). The use of phosphoric acid alone tended to associate with SCC ($p = 0.05698$). Only one personally interviewed farm manager (14.3%) had no information regarding the acid cleaning agent, which was used on the farm, three managers (42.9%) knew the name of the distributor, two (28.6%) could name the product but there was only one manager (14.3%), who could name the active ingredient. Thus, it can be concluded that farm managers have only vague information about the active ingredients of the cleaning products used on their farms.

The survey contained questions regarding the concentrations of caustics, acids, and disinfectants. In the surveyed dairies, the most used (38.9%) concentration for caustic solutions was 1% by far, while concentrations of 1.5 and 2% (min. 0.2%; max. 10%) were also frequently used, in 8.3 and 11.1% of the farms, respectively (Supplementary Table 8). The caustic concentration had no significant relationship with any of the studied milk production parameters ($p \geq 0.4369$). Four out of seven managers (57.1%) did not know the concentration of the caustic solutions, which were used on their farms, the other three (42.9%) said 2–3% concentration, which—based on the results of the questionnaire—was probably greater than the actual values; therefore, they were not aware of the exact numbers. However, all of them stated that they used them according to the product manuals.

The acids were mostly (40.0%) used in a concentration of 1% also, but concentrations of 0.5%; 2% and 5% (min. 0.2%; max. 10%) were also quite common: in 8.6, 11.4, and 8.8% of the farms, respectively (Supplementary Table 9). The acid concentration had no significant relationship with any of the studied milk production parameters ($p \geq 0.6543$). Five out of seven farm managers (71.4%) were not aware of the concentration of the acid solutions, which were used on their farms, one (14.3%) manager said 0.2% and another

TABLE 6 Relationships between Days in Milk (days) and pre- and post-milking disinfectants.

Variables	Layers	Mean	SD	*n*	*P*-value
Active ingredient of a pre-milking disinfectant	Alcohol	329	16	2	
	Chlorine	382	46	8	0.3464
	Chlorine/alcohol	394		1	0.1601
	Chlorine/iodine	350		1	0.3417
	Chlorine/lactic acid	ND			
	Lactic acid	393	34	4	0.1584
	Other acids	367		1	0.2278
Using chlorine as a pre-milking disinfectant	No	371	39	7	
	Yes	380	42	10	0.6770
Using lactic acid as a pre-milking disinfectant	No	371	42	13	
	Yes	393	34	4	0.6017
Using alcohol as a pre-milking disinfectant	No	382	39	14	
	Yes	350	40	3	0.6888
Active ingredient of a post-milking disinfectant	Chlorine	361	38	5	
	Iodine	351	22	5	0.2751
	Iodine/chlorine	360	14	2	0.9563
	Iodine/lactic acid	339	26	2	0.9430
	Iodine/lactic acid/chlorine	450		1	0.0808
	Iodine/other	ND		0	
	Lactic acid	399	24	4	0.3343
	Lactic acid/chlorine	411	62	2	0.4413
	Lactic acid/other	405	35	2	0.3813
	Other	452		1	0.1234
Using iodine as a post-milking disinfectant	No	385	44	11	
	Yes	368	42	14	0.9860
Using lactic acid as a post-milking disinfectant	No	370	42	14	
	Yes	383	44	11	0.5039

ND, no data.

0.3%, which—based on the results of the questionnaire—were probably lower than the actual ones. However, all of them stated that they used the acids according to the product manuals.

Out of 43 surveyed farms, only 17 (39.5%) reported the use of a separate disinfectant for the milking machines. Peracetic acid was the most used disinfectant (88.2%), and 29.4% of the dairies also reported the use of hydrogen peroxide ($n = 17$). The use of peracetic acid was significantly associated with the percentage share of marketed milk ($p = 0.0432$). The results of the personal interviews corresponded with the results of the questionnaires; four farm managers (57.1%) said that they did not use a separate disinfectant while three (42.9%) reported using peracetic acid. The disinfectants were used in concentrations between 0.1 and 10%, but usually (in 61.5% of the farms) between 0.3 and 2% ($n = 13$) (Supplementary Table 10). The concentration of disinfectant had a significant relationship with the percentage share of marketed milk ($p = 0.0356$). In the dairies, where a separate disinfectant was used, none of the farm managers was aware of its concentration, but they all stated that the disinfectants were used according to the product manuals.

The key drivers in the procurement of milking machine cleaning products were also surveyed, and the respondents evaluated the different characteristics of the cleaning products on a scale of 1–10 ("1" for the most important, "10" for the least important characteristic). Figure 6 shows that the price was the most decisive procurement factor for a milking machine cleaning product, followed by reliable supply and the type of the cleaning process (one/two-component). The sale discounts were considered as the least important factor in the procurement process, and the corrosive impact did not prove to be important either.

The results of the personal interviews partly complied with the findings of the survey, because according to the farm managers the most important milking machine cleaning product characteristics were efficacy (all seven managers), followed by good value/price ratio (85.7%), reliable supply (71.4%) and non-corrosive impact (57.1%). Every personally interviewed manager emphasized efficacy, which corresponds to the factors named "active ingredient" and "duration of activity" in the questionnaire and was found to be of moderate importance only

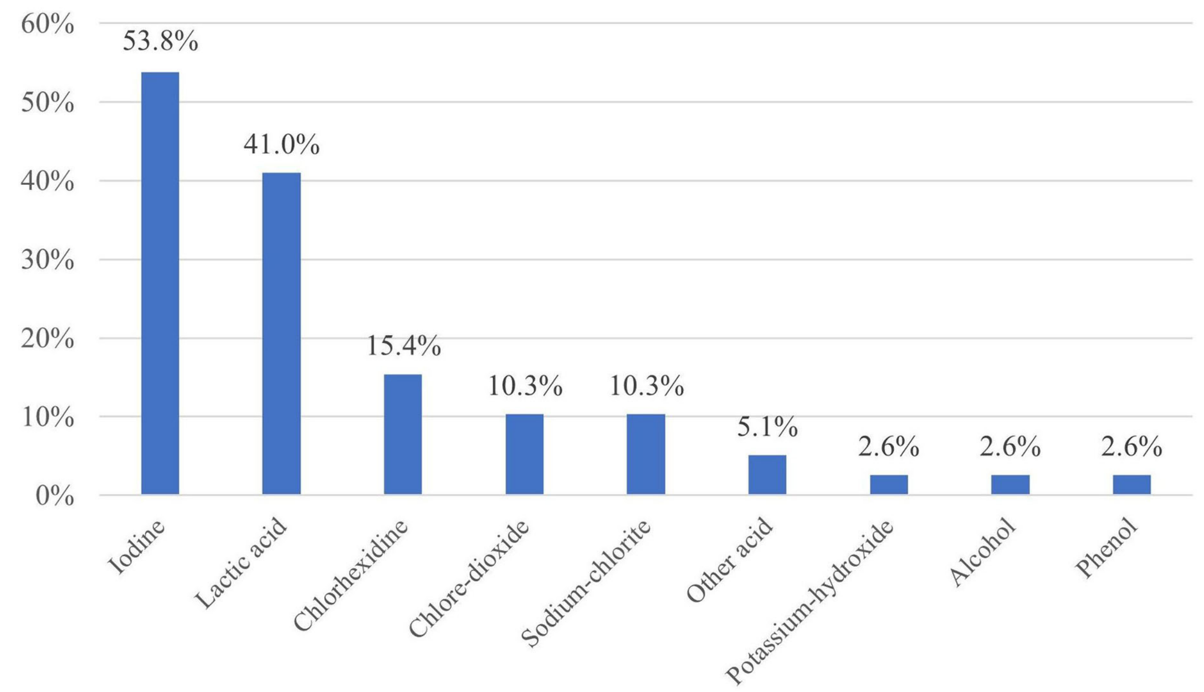

FIGURE 2
Active ingredients of the post-milking teat disinfectants ($n = 39$).

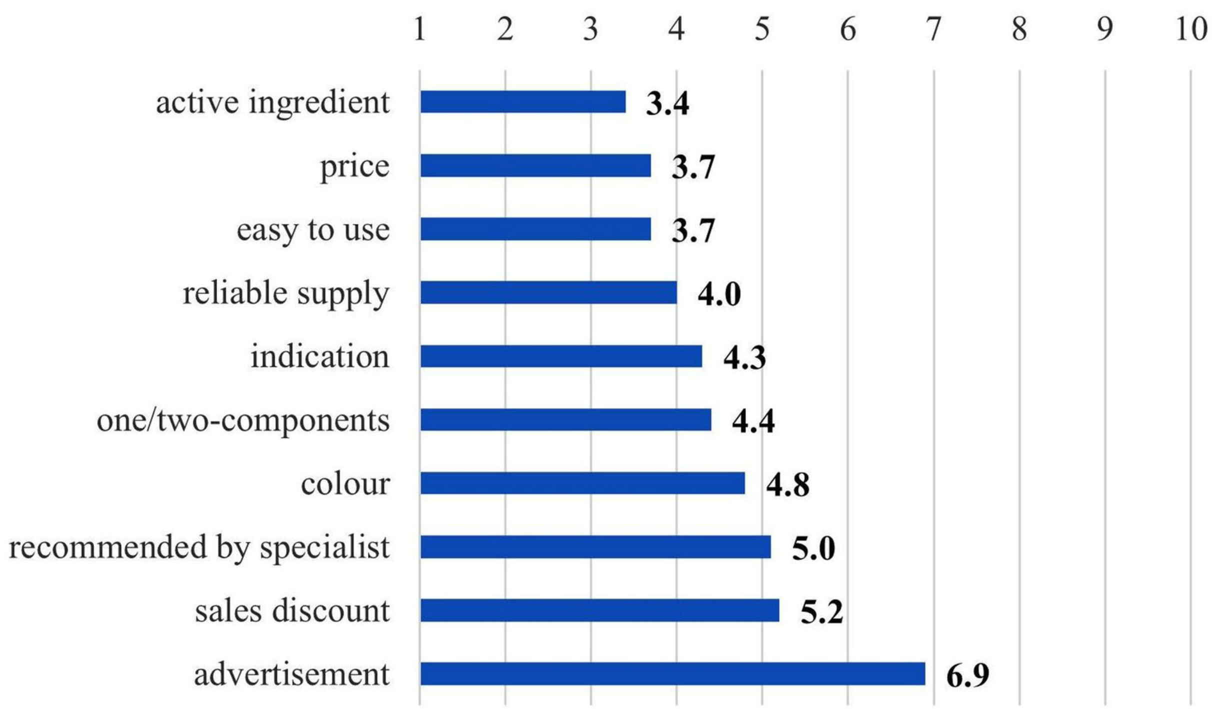

FIGURE 3
Importance of different procurement factors for pre- and post-milking disinfectants ($n = 43$).

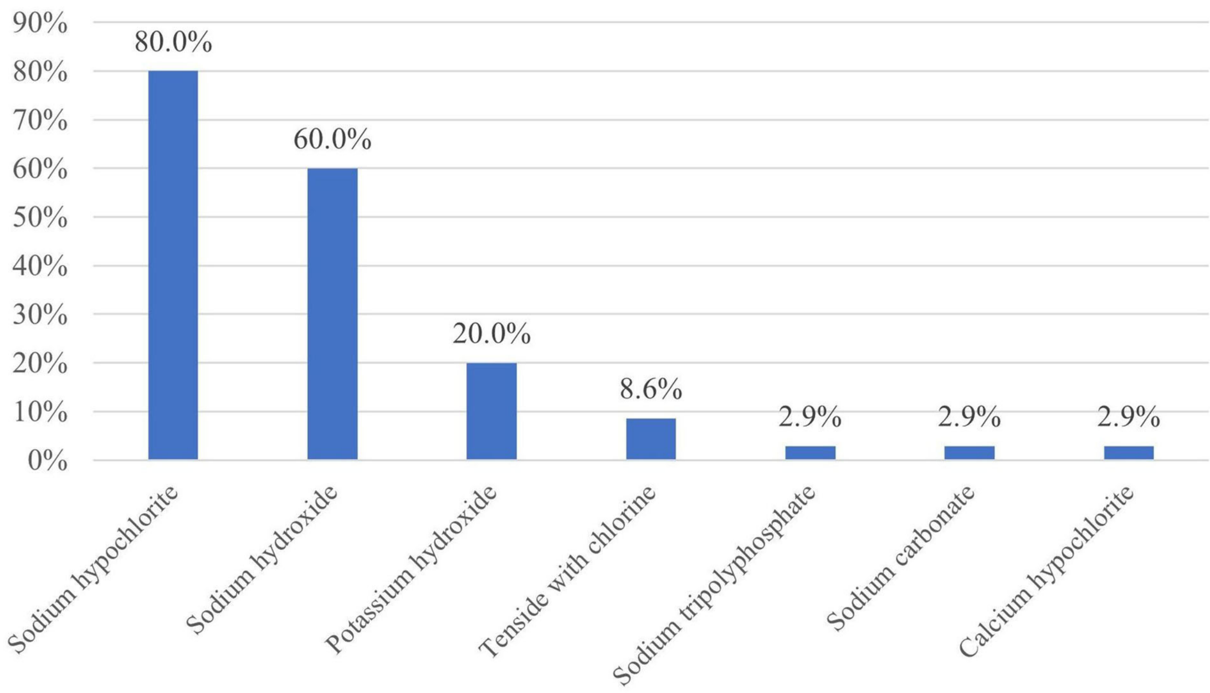

FIGURE 4
Active ingredients of the caustic detergents ($n = 35$).

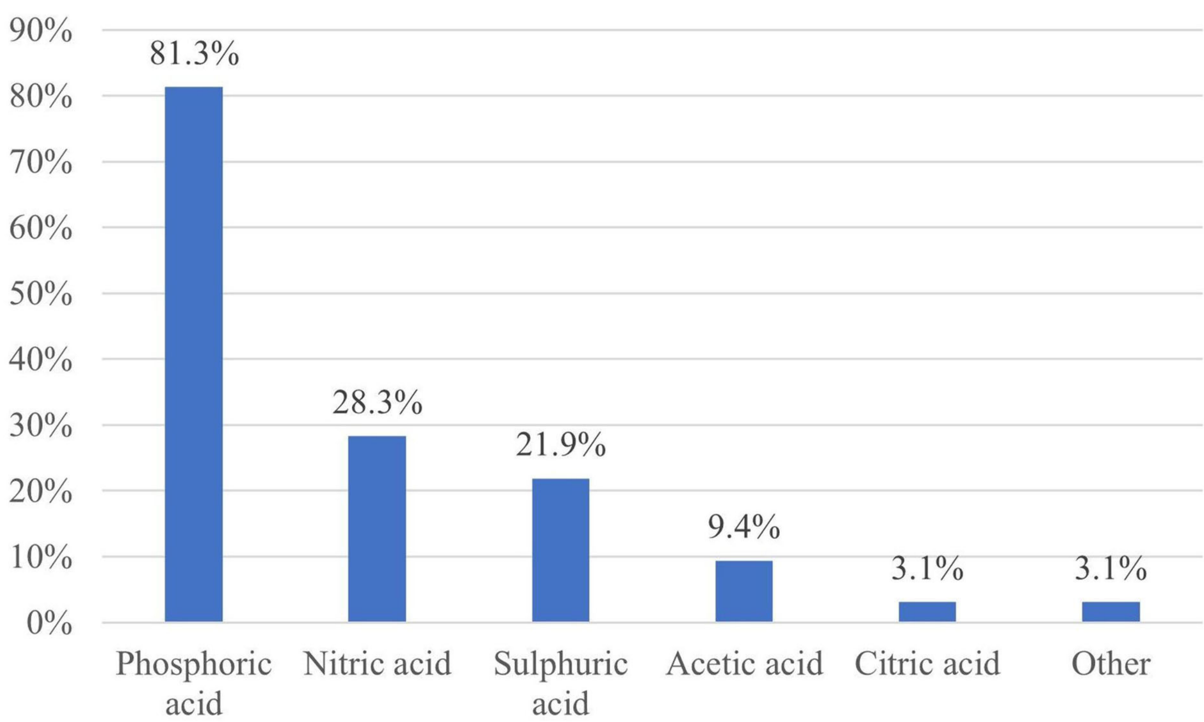

FIGURE 5
Active ingredients of the acid washes ($n = 32$).

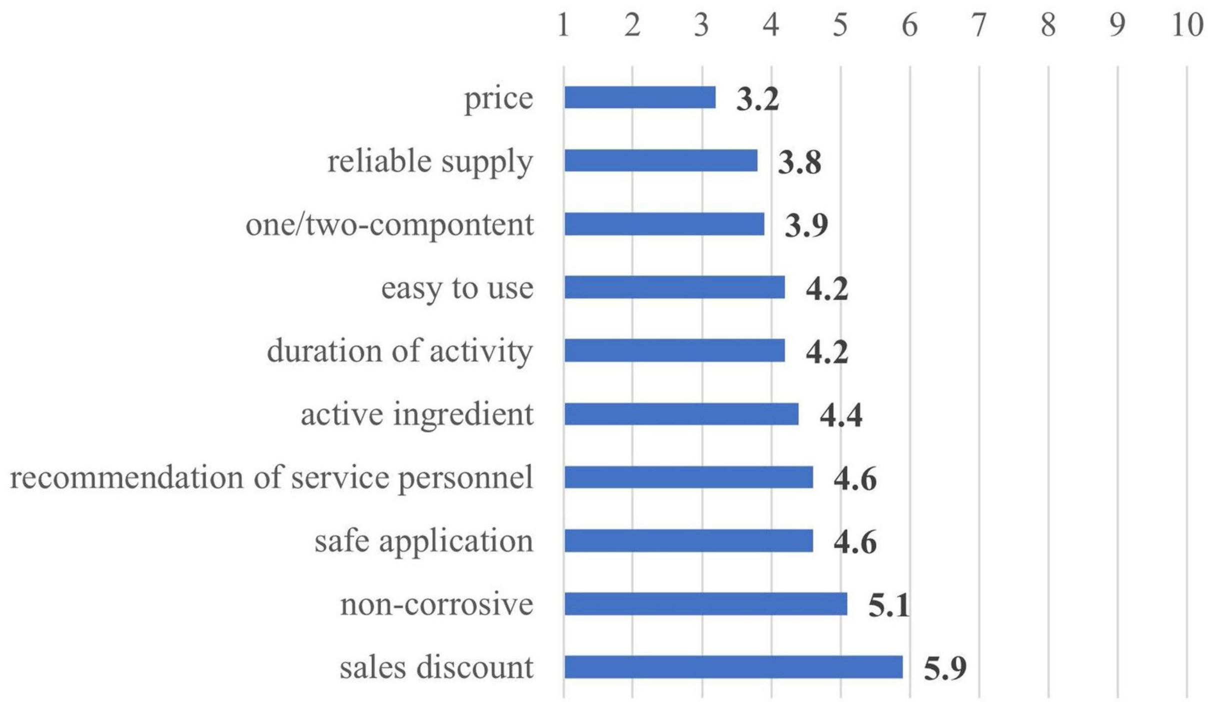

FIGURE 6
Importance of different procurement factors for milking machine cleaning products ($n = 38$).

in the survey. The reliable distributor, not damaging to teat cups and one/two-component product, was of far smaller importance (28.6% each). Other factors, namely, package size, easy to use, long shelf life, small per-unit cost, safe application, active at low temperatures, and no residues were mentioned once (14.3%) by the farm managers.

Discussion

Pre-milking teat disinfection

Effective pre-milking teat sanitation reduces the number of bacteria on teat skin, thus decreasing bacterial contamination of milk and improving milk quality (12, 13). It has been well-established that reducing teat end exposure to microorganisms can result in a reduced incidence of intramammary infection (14). Almost two-thirds of the surveyed farms (62.8%) used disinfectant teat dips prior to milking, but almost one-quarter of the farms (23.3%) still washed the udder with water. Washing with water is only indicated when the udder is very dirty, but it is not recommended as a routine part of udder preparation (15). The udder was wiped with dry paper towels in almost three-quarters of the farms (73.8%), which cannot play a role in spreading infection (16). A teat cleaning procedure that includes wet cleaning followed by manual drying with a paper towel will result in the lowest bacterial counts (4). In situations where herd infection levels are considered high and where the risk of spread of infection is greater, pre-milking disinfection of clean teats followed by teat drying can be beneficial. However, the routine application of pre-milking teat disinfectants in pasture-grazed herds is unlikely to be of benefit where herd SCC is below 200×10^3 cells/mL (17). In general, when cows were housed indoors, the procedure was found to reduce the incidence of new intramammary infection caused by environmental pathogens by >50% (17).

The pre-milking disinfectants containing compounds of chlorine are preferred in two-thirds (64.3%) of the surveyed commercial Holstein-Friesian herds, which complies with the international findings from Europe (16). The use of chlorohexidine digluconate is shown to have a significant efficacy against *Staphylococcus aureus* and *Streptococcus agalactiae* under experimental challenge conditions (17). The second most common pre-milking disinfectant was lactic acid in the surveyed Hungarian dairies (25.0%). The application of probiotic lactic acid bacteria (LAB) is now considered as the best choice for the treatment of many infectious human diseases and the control of bovine mastitis, and the use of probiotic LAB teat disinfectant as a protective barrier to inhibit pathogens and to improve the microbial balance of the teat proved to be beneficial (3). Therefore, the use of lactic acid as a pre-milking disinfectant might be further strengthened in dairy cattle farms to achieve a better microbial balance on the teat skin, thus, have a greater resistance against udder infections.

Post-milking teat disinfection

According to Oliver et al. (18), almost on every farm iodine is considered as the optimal active ingredient followed by lactic acid in post-milking disinfection, which can largely reduce the incidence of clinical mastitis. These findings correspond to the results of our survey which showed that 53.8% of the surveyed large Holstein-Friesian farms used iodine and 41.0% used lactic acid as active ingredients in their post-milking teat disinfection process, and the use of iodine and of lactic acid combined with other active ingredients resulted in significantly lower SCC. In the United States, iodine-based teat disinfectants are used in 66% of pre-milking and in 84% of post-milking disinfections in large dairy units, and the post-milking teat disinfection with barrier properties and higher free iodine content reduced the risk of clinical mastitis (4). Fitzpatrick et al. (19) stated that the most effective post-milking products to reduce the bacterial load on teat skin contained 0.6% diamine and 0.5% chlorhexidine, 0.6% diamine, and 0.5% w/w iodine in the case of staphylococcal, streptococcal, and coliform isolates, respectively. This is in harmony with other studies where iodine-based products were shown to be effective against a wide range of udder pathogens (20–22), although it should be emphasized that higher iodine concentrations may occur in milk by using these products, which might be an important food safety factor in infant formula production (23).

According to the personally interviewed farms managers, the drying impact on teat skin is an important characteristic of the teat disinfectants that should not dry the skin too much. This is an issue mainly with disinfectants containing iodine, as they can significantly dry the skin and manufacturers try to counterbalance this unwanted effect by using different additives. Chemical disinfectants can reduce the major pathogen infections, but the high concentration of chemical substances raised the concern of potential residues in milk (24). Therefore, during teat disinfections, the farm managers might be encouraged to use products with lower risk to human health. Introducing effective teat disinfectants with chemicals, which occur naturally in milk, is an opportunity in the udder health management, because concerns of residues in milk are minimized (2). Nevertheless, farm conditions and udder health management practices have a significant impact on the effectiveness of teat disinfection (21).

Milking machine cleaning

The efficacy of milking machine cleaning depends on the working solution content, the temperature of the water, and the application of sanitizer (18). Meanwhile, four out of seven (57.1%) personally interviewed farm managers considered three-phase cleaning systems better than five-phase ones, the five-phase cleaning systems were more common in the Holstein-Friesian dairy units, and it can be stated that the five-phase washing and disinfection are considered equally efficient, and the number of cleaning phases depends mainly on the specifications of the milking equipment manufacturers.

The typical automatic three-phase cleaning process can be divided into three different main phases: pre-rinse, washing phase, and post-rinse. During the washing phase, alkaline or acid detergents should be used. The alkaline detergent helps to remove organic deposits, such as milk protein and fat. Most detergents can work effectively at hot temperatures. Acid detergent is used periodically to remove mineral deposits from water and milk. The frequency of acid washing depends on the hardness of the water used on the farm (7). The number of acid descaling washes per week revealed a very diverse picture in our study: it ranged from once a week to 21 times a week, that is, after every milking, but most commonly it was performed daily or two to three times per week. The use of daily cold caustic cleaning in conjunction with daily hot acid cleaning or cleaning with a hot detergent/sanitizer twice a day maintained the lowest total bacterial count in milk and on plastic surfaces (18).

Sodium hypochlorite was the most preferred active ingredient of caustic detergents, 80.0% of the surveyed commercial dairy farms used it. While the addition of sodium hypochlorite to the pre-milking rinse water may have benefits in sanitizing internal milking equipment surfaces, it may also result in the formation of the residue of tri-chloromethane (TCM) in milk (25). Farms using cleaning products that contain a high sodium hypochlorite content (>8%) are highlighted as being more likely to result in TCM residues in bulk tank milk (26). The international agency for research on cancer states that TCM is possibly carcinogenic to humans (27). In the surveyed Holstein-Friesian dairies, the most used concentration of caustic solutions was 1% by far, while concentrations of 1.5 and 2% were also frequently used. A potential alternative sanitizer for milking machines might be peracetic acid. The addition of peracetic acid in the final rinse water and as a replacement for sodium hypochlorite would also reduce or eliminate TCM residues (5). 39.5% of the surveyed farms reported using a separate disinfectant for the milking machines which was peracetic acid in 88.2%. Peracetic acid was also more effective against *Prototheca zopfii* than sodium hypochlorite or iodine (28). A further potential alternative sanitizer is quaternary ammonium compounds (QAC) that are non-oxidizing surfactant-based disinfectants. Some of these compounds (diethyl ammonium chloride and dimethyl benzyl ammonium chloride) are now being promoted as an alternative to traditional sanitizer products; however, Gleeson et al. (5) found that the QAC product is not suitable for the cleaning of milking equipment due to the foaming effect of the product during the wash circulation.

Conclusions

A significant share (16.3%) of the surveyed Holstein-Friesian farms did not use any pre-milking disinfectants, which was still a considerable percentage, and applying pre-milking disinfection might improve the udder health in these dairy units. Many products of both pre- and post-milking teat disinfectants are available on the market, but compounds of chlorine are the most preferred active ingredients for pre-milking disinfectants while iodine in post-milking disinfection. Lactic acid is ranked second in both disinfections. The decision-makers on the farms prefer these active ingredients primarily because of their efficacy, which is the most decisive factor in buying teat disinfectants. Price and continuous, reliable supply are also important factors during procurement. Teat disinfectants that are colored, that is, easy to see on the teat skin, and consist of one component are also more preferred by the farm managers. Advertisements and sales discounts have no significant effect on the procurement of teat disinfectants.

The decision-makers on the Holstein-Friesian farms are not as well-informed regarding milking machine cleaning products (caustics and acids) and their use as they are as regards to teat disinfectants. In many cases, they are not aware of their active ingredients and/or concentrations, they only refer to the product manuals. Their opinions greatly differ on that how frequently an acid wash is needed. In some herds (11.9%), acid wash was performed after every milking, but more than half of the farms (52.4%) only run an acid wash every other day or even less frequently. Several milking machine cleaning products, both caustics and acids, are available on the market, but sodium hypochlorite and sodium hydroxide are the most preferred active ingredients of caustic detergents while phosphoric acid is the most used acidic product. Furthermore, 39.5% of the large commercial dairies used a separate milking machine disinfectant, in most cases, peracetic acid. The use of milking machine detergents is greatly influenced by the milking equipment because milking machine distributors often specify which sanitizers are allowed.

According to our knowledge, this was the first scientific, large-scale study assessing the use of teat disinfectants and milking machine cleaning products in Hungarian dairy cattle farms, but the limitation of the survey is the non-representative nature of the sample. As most of the teat disinfectants and milking machine cleaning products proved to be similarly effective in milk production, the use of those, which may pose less risks to human health and the environment, might be more encouraged in the future.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The revised survey was reviewed by the Scientific Research Committee of the Faculty of Veterinary Science, Budapest, and found exempt from human subject protection regulations. The participants provided their written informed consent to participate in this survey.

Author contributions

LÓ conceived and designed the study, collected the data, and acquired funding. DI developed the statistical models and analyzed the data. DI and LÓ contributed to the conceptualization and writing the paper. All authors contributed to manuscript revision, read and approved the submitted version.

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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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Supplementary material

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The application, value, and impact of outcomes research in animal health and veterinary medicine

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Outcomes research is a relatively recent field of study in animal health and veterinary medicine despite being well-established in human medicine. As the field of animal health is broad-ranging in terms of animal species, objectives, research methodologies, design, analysis, values, and outcomes, there is inherent versatility in the application and impact of the discipline of outcomes research to a variety of stakeholders. The major themes of outcomes relevant to the animal health industry have been distilled down to include, but are not limited to, health, production, economics, and marketing. An outcomes research approach considers an element of value along with an outcome of interest, setting it apart from traditional research approaches. Elements of value are determined by the stakeholders' use of products and/or services that meet or exceed functional, emotional, life-changing, and/or societal needs. Stakeholder perception of value depends on many factors such as the purpose of the animal (e.g., companion vs. food production) and the stakeholder's role (e.g., veterinarian, client, pet-owner, producer, consumer, government official, industry representative, policy holder). Key areas of application of outcomes research principles include comparative medicine, veterinary product development, and post-licensure evaluation of veterinary pharmaceuticals and/or biologics. Topics currently trending in human healthcare outcomes research, such as drug pricing, precision medicine, or the use of real-world evidence, offer novel and interesting perspectives for addressing themes common to the animal health sector. An approach that evaluates the benefits of practices and interventions to veterinary patients and society while maximizing outcomes is paramount to combating many current and future scientific challenges where feeding the world, caring for our aging companion animals, and implementing novel technologies in companion animal medicine and in production animal agriculture are at the forefront of our industry goals.

KEYWORDS
outcomes research, outcomes, animal health, veterinary medicine, veterinary research

Introduction

Outcomes research has been defined as research concerned with outcomes, or end results, and entails the application of clinical and population-based methods to optimize healthcare practices and interventions (1). Albeit considered a formal discipline in human medicine for several decades (2), it remains a relatively young area in veterinary medicine (3) and the animal health industry. Outcomes and value are key foundations of this field, with outcomes research being at the intersection of outcome optimization and quantification of the elements of value. Of recent relevance to animal health are outcomes such as quality of life (4), individual preference (5), and cost-effectiveness (6). Commonly, value is associated with a reduction in cost; however, cost and value are not always synonymous (7). Value can be represented by many factors that address functional, emotional, life-changing, and/or societal needs (7). The perceived value of those outcomes ultimately depends on the stakeholder, whether a pet-owner, farm manager, government official, or industry representative.

Currently, the veterinary and animal health sectors are facing substantial challenges including an increase in the pet population, increase in the costs of veterinary care and products, increased financial limitations of owners and managers, and pressure to increase food supply (8, 9). These challenges have exacerbated the ongoing reduction in access to large and companion animal veterinary care, especially in communities that already have inadequate access to veterinary services (10). We theorize that outcomes research principles and methods that consider client preferences, costs, and health risks can aid decision-making by veterinary practitioners and animal health professionals to minimize the use of clinical practices or interventions that are medically ineffective or not cost-effective while maximizing resources. In this perspective article, we briefly discuss the principles and methods of outcomes research, illustrate current examples of this field in the animal health sector, and describe the application of human healthcare outcome research trends and novel technologies in the animal health industry.

Brief introduction of principles and methods of outcomes research

The key themes of outcomes vital to the animal health industry can be distilled to health, production, economics, and marketing, although there are often points of overlap among them. Health and production outcomes are many and well-known in the companion and large animal veterinary fields. Varying based on the targeted species, economic outcomes in companion animals typically reflect cost to the pet-owner, willingness to pay, and overall affordability of veterinary care on an individual animal basis. As food animals are considered a commodity, livestock producers are most interested in an overall low cost and/or an increased net-return on investment. Marketing outcomes, instead, include any characteristic that can be used to market a product and/or service that demonstrates a clear value proposition (11), such as a more affordable alternative or a non-monetary metric. Measuring the safety and efficacy of administration of oral and topically administered fluralaner in canines with sarcoptic mange (12), demonstrating canine acceptance of two bioequivalent carprofen chewable tablets (13), or evaluating equine treat palatability and associated owner preferences (14), are examples of studies with marketing outcomes. Intuitively, economic outcomes commonly overlap with health, production, and marketing outcomes. For instance, the use of gamithromycin for metaphylaxis in stocker steers was associated with better performance measured by average daily gain (production outcome), lower morbidity (health outcome), and greater net-return per head (economic outcome) compared to the competitor product—ceftiofur crystalline free acid (marketing outcome) (15). Evaluating key outcomes and value relevant to the decision makers, while optimizing efficiency, demonstrating comparative efficacy, and maximizing resources demonstrates the quintessence of outcomes research.

The overarching goal of healthcare providers, either human or veterinary, should be to achieve projected outcomes and improve value for patients (16). Value, however, is not the same as cost. Instead, value refers to focusing on quality of service, as opposed to volume, and on maximizing outcomes that matter to patients relative to the incurred costs (16). Metrics of value are not mutually exclusive and multiple metrics may be represented within a study while addressing more than one stakeholder need. In fact, Almquist et al. (7), state that the more elements of value that are conveyed, "the greater a customer's loyalty and the higher the company's sustained revenue growth". The four types of needs—functional, emotional, life-changing, and societal (7)—used to represent value are determined by the stakeholder. Potential functional needs may include interventions and/or services that reduce time and/or effort (e.g., ease of administration), avoid hassle (e.g., acceptability, palatability), reduce risk of disease, or integrate easily into daily routines (7). Value can also address psychosocial or emotional needs such as improving quality of life, contributing to overall increase in wellness, providing therapeutic value, and being readily available to the stakeholder when needed. Lastly, life-changing and social impact needs that contribute to perceived value reflect aspects of a product/service that may provide hope or an organization that considers charity and gives back, respectively (7). Philanthropy efforts can be seen in the work done by The Zoetis Foundation which has committed to providing $35 million dollars over 5 years to support communities and their animals, veterinary training, veterinary student scholarships, and to care for animals impacted by disasters (17). Additional philanthropic efforts have been put

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forward by Elanco's Healthy Purpose™ initiative to advance the well-being of animals, people, and the planet (18), and the MerckHelps™ assistance program to provide Merck medicines and vaccines for free to people who qualify (19). A decision to purchase a product from these companies may be driven, in addition to the potential use of the product, by the customer's desire to make a difference.

For a review on the origins and evolution, as well as principles and application of outcomes research in veterinary medicine, the reader may refer to Cernicchiaro et al. (3).

Application of human healthcare outcome research trends and novel technologies in the animal health industry

Every year, the International Society for Pharmacoeconomics and Outcomes Research (ISPOR), the international organization for the advancement of policy, science and practice of the discipline of outcomes research, publishes the "Top 10 Health Economics and Outcomes Research (HEOR) Trends" (20). Though focused on human healthcare, these trends are also shaping the themes and the methodologies applied to the animal health industry, especially in the areas of drug and healthcare pricing, digital technologies and advanced analytics, which were identified among the 2022–23 top trends (20). Discussions pertaining to the cost and price of products and services have dominated the veterinary field for years. The price of drugs and transparency of costs incurred for veterinary treatment is essential for building trust with pet-owners (21) and providing a spectrum of care (22). As per the National Health Council (23) "value assessment advises whether a health service (e.g., drug, device, and surgery) should be used, and if so, how it is best used in the healthcare system, and which patients are most likely to benefit from it." Most value assessment frameworks evaluate the health benefits and risks of using a treatment or technology, but they can also assess costs and other wider impacts on a population (23). Several value assessment approaches, such as cost-effectiveness analysis, are used to inform product pricing. Cost-effectiveness models evaluate health effects and costs associated with treatment (24), hence offering a better option than a cost-benefit analysis, which simply monetizes a health effect while ignoring important aspects associated with treatment such as quality of life (24, 25). Similarly, decision analytical models, which provide evidence to guide decision making by utilizing mathematical techniques to synthesize data comparing expected costs and consequences of potential decisions (26), can be used to evaluate long-term outcomes as well as economic impacts (27). Survival extrapolation that includes general population mortality has been recently utilized (28) for evaluating oncology treatments in companion animals. While some of the trends observed in human and veterinary healthcare overlap, a disconnect in methodologies remains, and thus, a tremendous benefit could be gained by translating and applying these methods to current issues in animal health.

The use of digital technologies in animal health is also of recent interest for areas like willingness to pay for veterinary telemedicine (29) and assessing traceability of live animals and their products (30). The trend of precision medicine, or personalized medicine, is a growing field in human (20) but also in veterinary medicine. Precision medicine utilizes big data (20) with predictive technologies and advanced analytics to identify the best treatment on an individual basis. Recently, the use of technology demonstrated successful bovine respiratory disease treatment of individual cattle upon arrival that was similar in overall effect to traditional metaphylaxis (31). This predictive technology reduced antibiotic use in the cattle production environment after determining whether an individual animal required treatment or not (31) rather than a subjective decision to treat an entire population at the time of arrival; hence, technology has the potential to improve antimicrobial stewardship and subsequently reduce the costs associated with treatment (31).

Real-World Evidence (RWE) in decision making has prevailed as a top trend in recent years (20). The application of RWE by veterinary pharmaceutical companies to supplement the product development licensure process could be transformative in reducing the time to bring veterinary products to market. As defined by the Food and Drug Administration (FDA), Real-World Data (RWD) are data relating to patient health status and/or the delivery of healthcare, whereas RWE is the analysis of RWD regarding usage, benefits, and/or risk of a medical product (32). The advantages and limitations of the use of RWD and RWE in human healthcare product development have been reviewed in detail, and overall, show great promise to accelerate product development as the results and findings are more indicative of how the product performs in the real-world (33). As recently reported in human vaccine licensure (34), the use of RWD and RWE, could also expedite the regulatory approval process of vaccines and therapeutics in animal health, such as when using client-owned animals to prove concept of new animal drugs after the completion of necessary safety studies. A recently published guidance by the Food and Drug Administration, Center for Veterinary Medicine (FDA-CVM) describes how RWD and RWE can be used to support regulatory decisions relative to the effectiveness of new animal drugs (35). Despite having a greater external validity, there are concerns on relying solely on RWD and RWE for regulatory approval, as they lack the internal validity of randomized controlled trials. Likewise, not all objectives are amenable to RWE, including the development of pharmaceutical products against Biosafety level 3 and 4 pathogens, the production of novel compounds that are not yet made in mass production facilities, or measuring outcomes such as methane release or feed intake in dairy cattle. The abundance, diversity and size of RWE, which includes post-marketing studies, patient registries or physician reports, among other data sources, presents challenges to conventional analytic methods (20). In addition to knowledge translation and synthesis methods (e.g., systematic reviews, meta-analysis) which can assist the appraisal and synthesis of high volumes of data, machine learning techniques and artificial intelligence offer ways to analyze these data and provide clinical prediction and treatment, among other applications (36, 37). Early adoption of robust RWD collection and synthesis methods, coupled with the use of technology and automation would enable the timely generation, and at a reasonable cost, of stakeholder- and clinically-relevant outcomes in the veterinary pharmaceutical research and development, as well as in other relevant animal health areas.

Current examples and future directions of outcomes research in the animal health industry

The utility of outcomes research in the areas of comparative medicine, veterinary product development, and evaluation is demonstrated in the following sections.

Comparative medicine

In comparative medicine, human and veterinary medicine are considered as "two branches of one medicine", as they share similar problems and approaches to solutions for humans and animals (38). In human pharmaceutical and biological development, laboratory animal models are utilized to prove a concept, and to demonstrate efficacy, or safety, before utilizing non-human primate and/or human research subjects in clinical trials (39). Traditionally, murine models are used, however, inherent weaknesses and limitations have been demonstrated over the years with findings in rodents not translating well into human medicine—"mice are not men" (40). When choosing which animal model is most appropriate, there are scientific, regulatory, and animal welfare considerations to contemplate prior to designing research trials (40). In recent years, alternative laboratory animal models—including canine, feline, and swine models—have demonstrated extreme value in expediting advancements in human healthcare research and product development. Specifically, the use of purpose-bred canine and feline animal models has led to successful approval of many therapies for many rare, yet extremely debilitating and lethal, genetic diseases in humans (40, 41). These animals also offer more efficient and externally valid models than rodents for certain tumors, such as lymphoma and leukemia. These ailments are not only more common in canines than humans, they progress in the same aggressive manner (42), leading to the utilization of canines as pre-clinical models to evaluate new, and modify current, therapies for human hematopoietic neoplasia (42).

Animal models are also commonly used in the development of human and animal vaccines for infectious disease prevention and management (43). Swine have demonstrated their usefulness as a biomedical model for humans in terms of metabolic, cardiovascular, digestive, and bone diseases (44–47). Lelovas and colleagues (44), discussed lessons learned over the last two decades of cardiovascular research utilizing animal models and the comparative anatomy of swine to humans, deeming minipigs to be the most appropriate animals for cardiovascular research. Commercial swine have also been used in biomedical research, most commonly in nutrition and physiology studies, where growing swine proved a suitable model for human metabolic studies in food research (45).

The longevity of companion animals has been substantially extended thanks to the improvements made in veterinary care as well as the advances in dietary and medical technologies. Attempts to understand the phenomenon of aging, as a way to increase the healthy lifespan of people and animals, has led to innovative initiatives such as the Dog Aging Project (48), which aims to understand how genetic, lifestyle, and environmental determinants influence aging, and how that information can be used to inform medical breakthroughs for humans and dogs. McCune and Promislow (49) suggested that dogs, given their proximity to humans in terms of genetic variation, pathophysiological processes, shared environment, and also because of their relatively short lifespan, can serve as sentinels for observing the beneficial and detrimental effects of environmental factors. Maximizing healthy aging and quality of life outcomes, as well as advancing efficacious, safe and affordable treatments and services for aging pets should become future priorities of the veterinary and animal health sectors.

Outcomes research in comparative medicine utilizes animal models for veterinary and human medicine evaluation, refinement, and ultimately approval of human and veterinary therapies and interventions, and considers relevant outcomes addressing primarily *functional* and *emotional* values, leading the way toward an overall improvement of the quality and longevity of human and animal life.

Veterinary product development, licensure, and post-licensure evaluation

In the veterinary field, pharmaceutical products and biologicals rely solely on research to provide pivotal information under regulatory guidance in order to receive approval for licensure from the respective agencies (50). Traditionally, the product development and licensure processes require many different experimental trials to demonstrate proof of concept, efficacy, and safety. It is estimated that the time required to develop a veterinary pharmaceutical product is 5–15 years, from discovery to licensure, with costs potentially exceeding $100 million (50). Conversely, the time to develop and license a veterinary vaccine is ∼5–8 years with costs estimated between $50 and $100 million (50). Time to licensure is generally shorter for veterinary products compared to human products, and the input costs and profits are also reduced. The COVID-19 pandemic highlighted the ability to rapidly develop a safe and effective human vaccine using mRNA technology (20). Although this example was an unusual pathway to market which emerged under extreme conditions, this rapid time to market amidst a global emergency shows there is room to improve efficiency in the licensure process for veterinary and human medicine fields moving forward. Post-licensure evaluation is commonly conducted to compare against other licensed products to yield marketing information and/or to supplement technical bulletins; however, additional time, labor, expertise, animals, and costs are required to generate supplemental data to prove value and the competitive edge over rival products. A major difference between human and animal health development/approval is that because of the importance of being approved/listed in insurance/formularies (i.e., market access), when human health products go through the approval process, they include a comprehensive assessment of cost/value, which is traditionally not implemented in animal health (unless a company wishes to do so post-approval). Through the use of outcomes research, the product development and licensure pathways have the potential to more efficiently utilize resources by addressing efficacy or effectiveness outcomes, as well as added value such as ease of administration, storage or temperature requirements, affordability, and/or net-return on investment.

Discussion

Outcomes research has been a formal discipline for over two decades, although more formally implemented in the animal health industry in recent years. The foundation of outcomes research is comprised of two elements, outcomes and value. This added component of value is what sets the discipline of outcomes research apart. Value can be measured by many characteristics that satisfy four primary needs: emotional, functional, societal, and life-changing, with the perceived value being determined by the stakeholder. Although decision-makers/stakeholders and values can differ in animal health, human health outcomes research continues to pave the way through shaping outcomes research based on current trends, and through the development and implementation of novel methodologies. Though easily transferable to the animal health industry, work is still needed in interpreting novel technologies utilized in human healthcare, such as long-term cost-effectiveness and decision analytical models. Additionally, the use of Real-World Data and Real-World Evidence to aid and/or supplement in the product licensure process in animal health could transform veterinary product development. While underrepresented in the literature during development due to confidentiality and intellectual property issues, outcomes research plays a role in licensure of veterinary products during the development phase as well as post-licensure as the focus of marketing veterinary products is through comparative efficacy and value proposition. Future efforts of outcomes research in the animal health industry should focus on bridging the gap between novel methodologies and technologies utilized in human health and translating their efforts into the context of animal health.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

DD and NC conceived and outlined and wrote the manuscript. DR, BB, KD, and MM contributed to the article, reviewed, and approved the final version of the manuscript.

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Conflict of interest

Author KD was employed by Latham BioPharm Group. Author MM was employed by Zoetis.

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.

Publisher's note

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Prevalence of methicillin-resistant *Staphylococcus aureus* in dairy farms: A systematic review and meta-analysis

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is an opportunistic bacterium that causes many human and animal infections worldwide. MRSA infections are classified as priority infections owing to their high morbidity and mortality, with a significant risk of zoonotic transmission. This study aimed to determine the pooled prevalence of MRSA in dairy cattle farms and its heterogeneity. Relevant studies were retrieved from three databases: PubMed, Web of Science, and Scopus. The pooled prevalence of MRSA in dairy farms was estimated using a random-effects model. Subgroup and meta-regression analyses were used to assess the probable sources of heterogeneity. Sensitivity and publication bias analyses were also performed. A total of 94 articles were eligible for inclusion in this meta-analysis. The pooled prevalence of MRSA was estimated to be 3.81% [95% confidence interval (95% CI) = 2.61–5.20] with significantly high heterogeneity (I^2 = 96.6%, p = 0.00). For the subgroup analysis among continents, the prevalence was highest in Asia (4.89%; 95% CI = 2.88–7.35) and lowest in South America (1.33%, 95% CI = 0.00–5.49). As for the year of publication, MRSA prevalence was highest in reports published from 2015 to 2018 (4.36%, 95% CI = 2.41–6.80) and lowest in reports published before 2015 (2.65%, 95% CI = 0.75–5.52). As for sample type, the prevalence of MRSA in cattle milk (3.91%, 95% CI = 2.64–5.39) was higher than that in other sample types (1.19%, 95% CI = 0.05–3.24). These three factors were not significantly associated with the pooled prevalence of MRSA (p > 0.05). Therefore, the findings of this study indicate that the prevalence of MRSA has been minimal and consistent in dairy cattle farms over time.

KEYWORDS

methicillin-resistant *Staphylococcus aureus*, MRSA, meta-analysis, systematic review, dairy cattle farm

Introduction

Staphylococcus aureus is a commensal bacterium that can be found on the skin, mucous membranes, and upper respiratory tracts of both animals and humans (1). However, it can be an opportunistic pathogen that causes various infectious illnesses in humans and animals (2). *S. aureus* is associated with many human disorders, from skin and soft tissue infections to life-threatening septicemia (3). In veterinary medicine, it is a common cause of bovine mastitis in dairy cattle, resulting in high economic losses worldwide (4).

Methicillin-resistant *S. aureus* (MRSA) was first documented in 1961 (5). MRSA strains were phenotypically identified using cefoxitin and oxacillin susceptibility assays (6). The gold standard for detecting MRSA is through the detection of the *mecA* gene, which encodes a protein called PBP2a, that has a poor affinity for β-lactam drugs, resulting in resistance to methicillin (7, 8). According to the recorded data, methicillin resistance has been identified in 50–70% of *S. aureus* strains isolated from the hospital environment, causing ∼100,000 infections in the United States each year, with a 20% mortality rate (9). In 1972, MRSA was first reported in domestic animals as a pathogen causing bovine mastitis in dairy cattle in Belgium (10). Since then, various studies reported the zoonotic transmission of MRSA from livestock, especially pigs, poultry, and cattle, to farm workers and exposed people, which has been known as livestock-associated MRSA (LA-MRSA) (11–13). The majority of LA-MRSA isolates lack toxins such as PVL and enterotoxins (14) and are reported to have multiple antimicrobial resistance (15).

In the past two decades, numerous studies have reported different prevalence rates of MRSA on dairy cattle farms in different regions. These variations might be associated with isolation protocols, farm management systems, sample sizes, sample sources, and other factors (16). Most studies have detected MRSA in bovine mastitis cases. However, several studies have demonstrated the presence of MRSA in raw milk, farm workers, and dairy cattle farms, indicating the possible risk of MRSA transmission within dairy cattle farms and across the dairy supply chain to the general public (17–19). Hence, the objective of this study was to estimate the global prevalence of MRSA isolated from various sample sources in dairy cattle farms through a systematic review and meta-analysis of published articles.

Materials and methods

Search strategies

The Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) guidelines were adopted for this study. Relevant studies published until 31 December 2021 were retrieved from three online databases: PubMed, Scopus, and Web of Science. The search was limited to original articles published in English. The keywords used for searching the relevant studies were "MRSA" OR "Methicillin-resistant *Staphylococcus aureus*" AND "dairy cattle" OR "dairy cow."

Inclusion and exclusion criteria

All original publications reporting the prevalence of MRSA, as determined by the detection of *mecA* and/or *mecC* genes, in dairy cattle farms were considered for analysis. The inclusion criteria were observational, cross-sectional, and case–control studies that determined the prevalence of MRSA from any sample source in dairy cattle farms. Studies were excluded from the analysis if they were (1) review articles, (2) experimental studies, (3) not written in English, (4) lack of a clear report on the prevalence of MRSA from any sample sources in dairy cattle farms, (5) lack of clear sample size, (6) performed on archived isolates, (7) no full text available, and (8) studies that used only phenotypic tests to detect MRSA. The titles and abstracts of the retrieved studies were evaluated for eligibility. After title and abstract screening, the full text of each article was thoroughly reviewed for inclusion. Two authors, SK and SB, independently performed study screening and selection. Disagreements were resolved through discussion.

Data extraction

Two authors individually extracted data from all included studies. Discrepancies between the data obtained by these two authors were discussed with a third author for consensus to avoid bias. The extracted data included (1) the name of the author and year of publication, (2) the continent where the study was conducted, (3) sample size, (4) sources of samples, (5) the number of *S. aureus* isolates, (6) the number of MRSA isolates, and (7) the detection method used.

Study quality assessment

The quality assessment criteria derived from Ding et al. (20) were used to evaluate the quality of the included studies. The checklist for determining the quality of studies consisted of these five questions: (1) Was the research objective clearly stated? (2) Was the sampling method described? (3) Was the study period and location clearly stated? (4) Were the examination methods and procedures for MRSA detection described clearly? (5) Were the samples clearly classified into different subgroups? The answers to each question were scored as "2" for yes, "0" for no, or "1" for unsure. A summation of the scores from all five questions was calculated, and the overall quality of each study was evaluated.

Statistical analysis

Meta-analysis was performed using the R package meta in the statistics software R (21, 22). The prevalence of MRSA in dairy cattle farms was determined by dividing the number of MRSA isolates by the total sample size. Because several studies reported zero prevalence of MRSA, Freeman–Tukey double arcsine transformation was performed for all raw proportions before conducting the meta-analyses to avoid excluding these studies (23). The classic meta-analysis model utilizing logit-transformed proportions and the corresponding standard errors in the inverse variance method was used to pool studies (24). Back-transformation of all estimated pooled prevalence was performed for ease of interpretation.

A random-effects model was used to estimate the overall pooled prevalence of MRSA in dairy cattle farms, together with its 95% confidence interval (95% CI). Cochran's Q-test was used to determine the heterogeneity of the pooled prevalence. Furthermore, the I^2 statistic was used to characterize the degree of heterogeneity across studies, with values of 25, 50, and 75% indicating low, medium, and high heterogeneity, respectively (25).

The subgroups in each study were used as the unit of analysis for all subgroup meta-analyses. Subgroup analyses were performed to investigate the heterogeneity between three variables: year of publication, continent, and sample type. The year of publication for each study was categorized into three groups consisting of "before 2015," "2015–2018," and "after 2018." Each study was classified into five continents: "Asia," "Africa," "Europe," "South America," and "North America." Sample type, referring to the sources of samples, was analyzed as two subgroups: "cattle milk" and "others." The "cattle milk" category included quarter milk, composite milk, bulk tank milk, and milk from clinical and subclinical mastitis cases. Other sources of samples, such as cattle nasal swabs, human samples, and environmental samples collected from dairy cattle farms, were included in the "others" category.

Meta-regression analyses were performed to investigate the significance of the between-study heterogeneity associated with three independent variables: year of publication, continent, and sample type. Levels within each independent variable were similar to those described for the subgroup meta-analyses. A univariate meta-regression model was created to determine the association between each independent variable and the prevalence of MRSA in dairy cattle farms. Furthermore, variables with $p \leq 0.25$ in the univariable meta-regression analysis were introduced to the random-effects multivariable meta-regression model.

Publication bias was examined using a funnel plot and Egger's test, where a $p < 0.05$ indicates statistical significance (26). The robustness of the results was evaluated using two sensitivity analyses. The first is a comparison of the results obtained from the random-effects and fixed-effects models. In addition, a leave-one-out meta-analysis was performed to evaluate whether any single study affected the results.

Results

Search results and study selection

A total of 2,601 records were identified from the three databases searched. These records consisted of 69 from PubMed, 2,446 from Scopus, and 86 from Web of Science. Of these, 155 records were duplicates and were removed before screening the titles and abstracts. After the screening process, 272 articles were included in the full-text review for inclusion criteria. Finally, 94 studies met the inclusion criteria and were included in the meta-analysis (16, 27–118). The remaining 178 articles were excluded for the following reasons: lack of clear prevalence of MRSA from any sample sources in dairy cattle farms ($n = 47$), lack of clear sample size ($n = 13$), archived isolates ($n = 56$), not written in English ($n = 9$), and studies that used only phenotypic tests to detect MRSA ($n = 53$), as shown in Figure 1.

Characteristics of the included studies

The 94 studies considered in this review were published between 2003 and 2021, with the majority published after 2018 ($n = 38$). These studies reported the prevalence of MRSA in 30 countries across five continents. Most studies were conducted in Asia ($n = 43$), followed by Africa ($n = 20$) and Europe ($n = 20$). The majority of studies reported MRSA detection in milk samples ($n = 90$), whereas only 22 studies reported the presence of MRSA from other sample types. The mean ± standard deviation of quality scores of all included studies was 7.91 ± 1.62, with a range from 4 to 10. The characteristics of the selected studies are shown in Supplementary Table 1.

Overall pooled prevalence of MRSA in dairy cattle farms

After data extraction, a total of 1,251 MRSA strains isolated from 47,236 samples collected from dairy cattle farms worldwide were included in the meta-analysis. As estimated from the random-effects model, the overall pooled prevalence of MRSA in dairy cattle farms was 3.81% (95% CI = 2.61–5.20), with high heterogeneity ($Q = 2773.64$; $I^2 = 96.6\%$; $p = 0.00$). These data are shown in Table 1 and Figure 2.

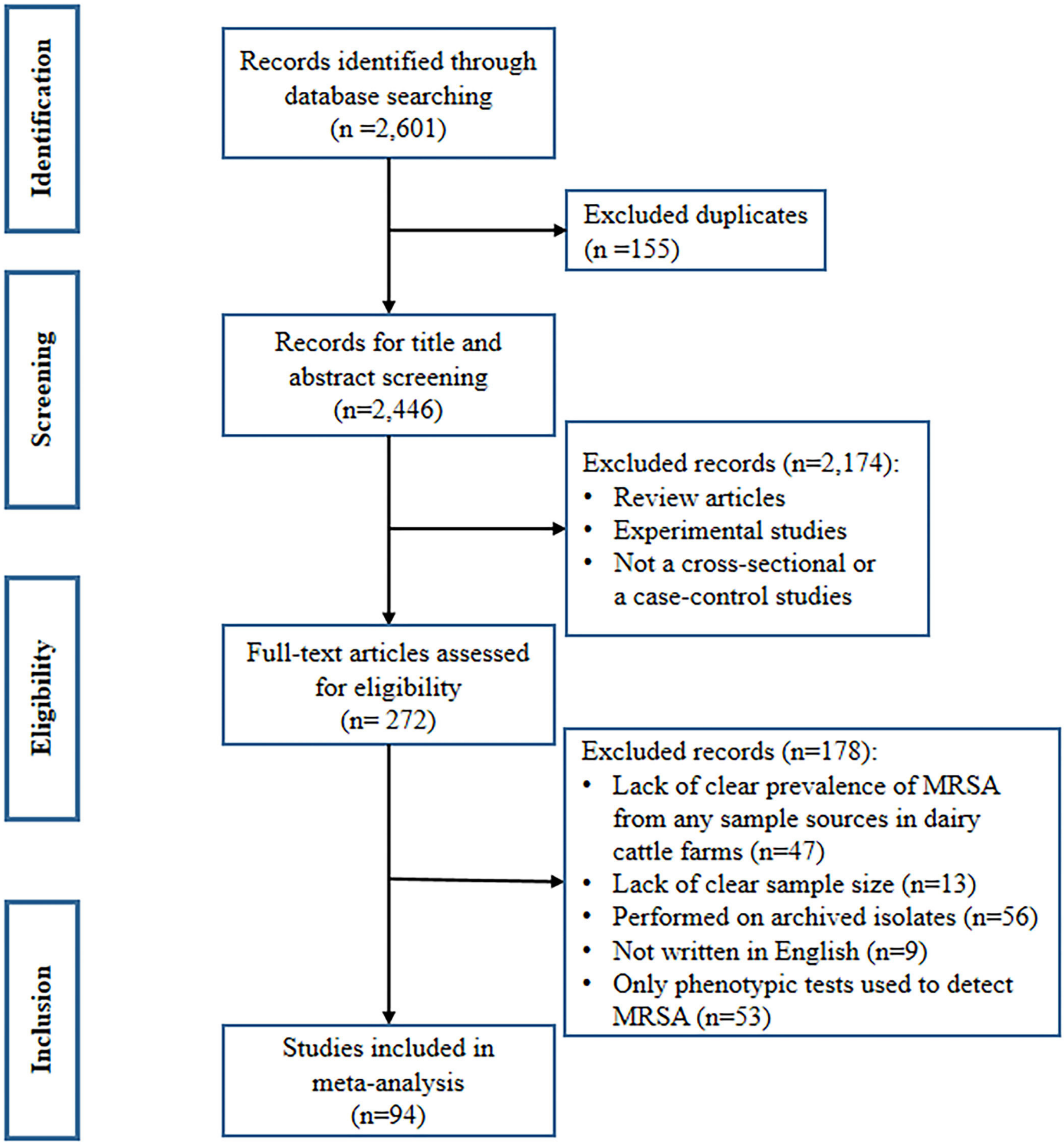

FIGURE 1
PRISMA flow diagram describing the selection process of the included studies.

Subgroup analysis and meta-regression analysis

The pooled prevalence of MRSA and the 95% CI for different subgroups of the year of publication, continent, and sample type are shown in Table 1. High heterogeneity was observed among all the tested subgroups. However, no statistically significant differences were detected between these subgroups. According to the year of publication, no significant trend in MRSA prevalence was observed, but the highest prevalence was observed among studies published between 2015 and 2018 (4.36%, 95% CI = 2.41–6.80). The pooled prevalence of MRSA in Asia appeared to be highest (4.89%, 95% CI = 2.88–7.35), followed by Africa (3.92%, 95% CI = 1.79–6.76) and Europe (3.19%, 95% CI = 0.99–6.38). The estimated prevalence of MRSA was lowest in South America (1.33%,

TABLE 1 Meta-analysis of methicillin-resistant *Staphylococcus aureus* (MRSA) prevalence from dairy cattle farms.

Subgroups	No. of studies or subgroups	MRSA prevalence (%)		Heterogeneity			*p*-values for subgroup differences
		Estimate	95% CI	Q	*p*	I^2	
Overall	94	3.81	2.61–5.20	2,773.64	0	96.6%	
Publication year							0.558
Before 2015	19	2.65	0.75–5.52	458.14	<0.01	96.1%	
2015 to 2018	37	4.36	2.41–6.80	1,245.34	<0.01	97.1%	
After 2018	38	3.94	2.10–6.28	675.93	<0.01	94.5%	
Continent							0.307
Africa	20	3.92	1.79–6.76	303.18	<0.01	93.7%	
Asia	43	4.89	2.88–7.35	1,727.65	0	97.6%	
Europe	20	3.19	0.99–6.38	392.32	<0.01	95.2%	
North America	3	1.61	0.02–5.05	12.12	<0.01	83.5%	
South America	8	1.33	0.00–5.49	176.50	<0.01	96.0%	
Sample type							0.318
Cattle milk	90	3.91	2.64–5.39	2,692.99	0	96.7%	
Others	22	1.19	0.05–3.24	102.21	<0.01	79.5%	

CI, confidence interval; Q, Cochran's Q-test for heterogeneity; I^2, I^2 statistic estimating the degree of heterogeneity across studies.

95% CI = 0.00–5.49). The pooled prevalence of MRSA in cattle milk (3.91%, 95% CI = 2.64–5.39) was higher than, but not statistically significantly different from, those in other sample types from dairy cattle farms (1.19%, 95% CI = 0.05–3.24). When the meta-regression models were analyzed for all three variables, no significant variable was associated with the heterogeneity of the overall pooled prevalence of MRSA in dairy cattle farms (Supplementary Tables 2, 3).

Publication bias and sensitivity analysis

The funnel plot created from the data obtained from the selected studies demonstrated asymmetry of distribution, as shown in Figure 3, indicating a publication bias among the selected studies. To investigate the sources of funnel plot asymmetry, the results from Egger's test showed a statistically significant coefficient bias (5.30 ± 0.77, $p < 0.0001$), revealing evidence of small-study effects. Furthermore, sensitivity analysis was performed to assess the robustness of the models used to estimate the pooled prevalence of MRSA. The overall pooled prevalence of MRSA in dairy cattle farms using a fixed-effects model was much lower than that using a random-effects model, as shown in Table 2. In addition, a leave-one-out meta-analysis was performed to investigate the impact of each study on the pooled prevalence of MRSA in dairy cattle farms. Removing the studies with the lowest or highest prevalence did not significantly influence the overall pooled prevalence of MRSA in dairy cattle farms, as shown in Table 2.

Discussion

The current study revealed that the global prevalence of MRSA isolated from various sample sources in dairy cattle farms, using a random-effects meta-analysis model, was 3.81%. Recently, Zaatout and Hezil (119) reported the global prevalence of MRSA isolated from bovine mastitis cases using a meta-analysis. Their reported prevalence was 4.30%, which was similar to our findings. The small variation in the estimated prevalence could be due to the fact that the current study included data from various sample types presented in dairy cattle farms, while the study by Zaatout and Hezil only selected reports from MRSA in the milk of clinical and subclinical bovine mastitis cases. We included data from a broad range of sample types to demonstrate the overall pooled prevalence of MRSA in dairy cattle farms, which can be used to determine the risk of MRSA transmission and contamination between cattle, humans, and the environment within the farms, and between the farms and other population at risk, especially the dairy consumers.

Subgroup analyses were carried out depending on the year of publication, continent, and sample type. We observed that the number of selected articles published before 2015 was limited (19/94). Increased numbers of studies were observed from 2015 to 2018 (37/94) and after 2018 (38/94). The highest pooled prevalence of MRSA was observed from 2015 to 2018 (4.36%) but not statistically different from that before 2015 and after 2018. In contrast, a recent meta-analysis on MRSA associated with bovine mastitis reported a significantly increasing trend in prevalence by the year of publication and suggested that it might

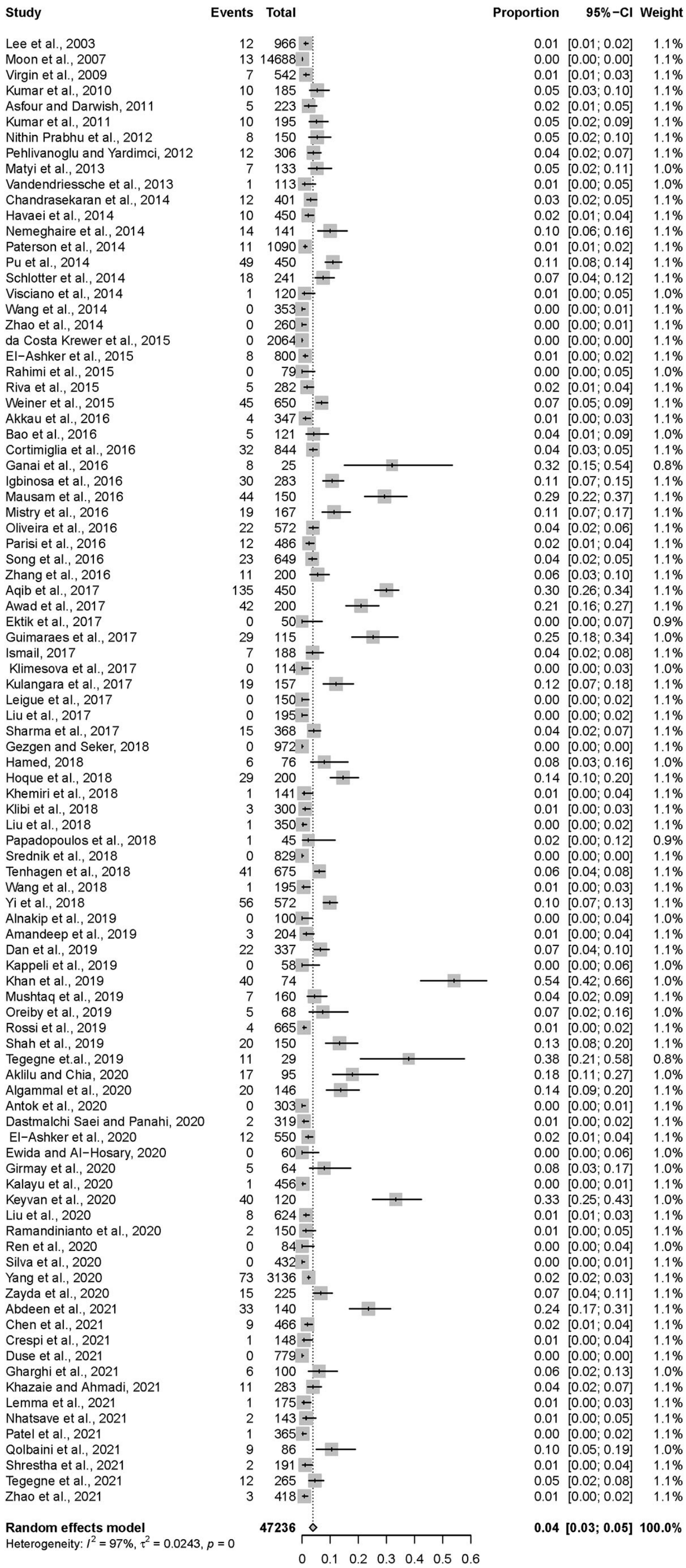

FIGURE 2
Forest plot demonstrating the pooled prevalence of MRSA in dairy cattle farms and its 95% confidence interval estimated by a random-effects model.

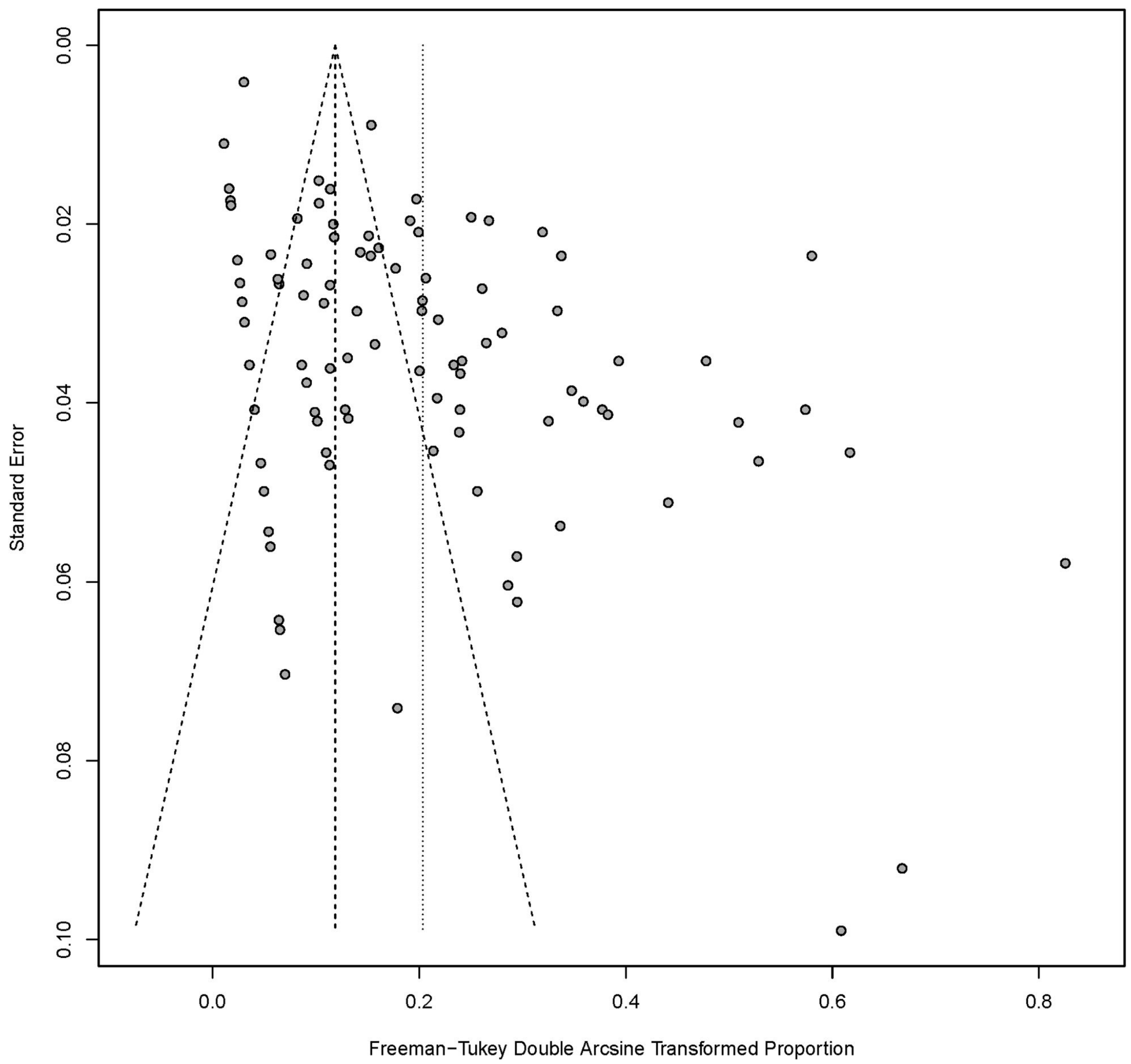

FIGURE 3
Funnel plot of data from all included studies examining the publication bias.

be influenced by the advancements in the detection methods used (119). This contrast can be explained by the difference in included studies, the different categorization used to create levels for the subgroup analysis of the publication year, and the different sample types to be included in both studies. All of these differences might be resulted in narrower CIs of the reported prevalence of each level of year of publication in the previous study, compared to those of reported prevalence in the current study. The narrower CIs could be potentially associated with the statistical significance observed in the previous study. The changes in prevalence emphasize the importance of monitoring MRSA in dairy cattle farms to assess the progress or success of any implemented antimicrobial resistance control program.

Although we could not demonstrate a statistically significant difference in pooled prevalence among subgroups, our results showed a substantially higher prevalence of MRSA in dairy cattle farms in Asia (4.89%) than in South America (1.33%). The milk production and dairy animal population in Asia have been increasing (120). During the period from 2010 to 2020, cow milk production in Asia increased up to 4.2%, which was the highest growth compared to other regions of the world (121). This could be associated with the high number of research studies investigating the presence of any zoonotic pathogens, especially MRSA, in dairy cattle farms. The limited number of publications in South and North America can potentially lead to an underestimation of the prevalence of MRSA in dairy cattle farms in these regions and should be noted. Moreover, the higher prevalence of MRSA in Asia compared to other regions might be due to the high consumption of antimicrobial agents in food animals (122), which could be related to the increased dairy

TABLE 2 Sensitivity analysis to determine the robustness of the results obtained from the models used.

Categories	No. of studies or subgroups	Prevalence (%)	
		Estimate	95% confidence interval
Model			
Fixed effects	94	1.12	1.01–1.22
Random effects	94	3.81	2.61–5.20
Leave-one-out analysis			
The lowest prevalence[a]	93	3.55	2.47–4.80
The highest prevalence[b]	93	3.89	2.68–5.30

[a]The study by Khan et al. (86) was excluded from the meta-analysis.
[b]The study by da Costa Krewer et al. (46) was excluded from the meta-analysis.

cattle population in this region and the available antimicrobial agents used in the region as they have a different selective pressure on MRSA. This phenomenon can also be attributed to the unethical use of antibiotics, especially in developing countries, where drugs are administered on the spur of the moment and without veterinarian monitoring (123). Another concern is poor farm sanitation and water management, both of which can facilitate MRSA transmission from animals to humans and vice versa and the development of antimicrobial resistance (124).

In addition to cattle milk, MRSA has been isolated from farm workers, farm environments, and other cattle organs. The pooled prevalence of MRSA in milk samples was lower than that in other sample sources from dairy cattle farms. Most milk samples reported in the selected studies were quarter milk samples collected aseptically; therefore, MRSA detected in cattle milk is generally a representative pathogenic strain of MRSA associated with intramammary infection and/or mastitis in cattle. In contrast, MRSA isolated from other sample sources could be either pathogenic or non-pathogenic strains, or a mix of both. Our findings suggest that MRSA is higher prevalent among bovine mastitis-causing *S. aureus* than other pathogenic or non-pathogenic *S. aureus* found in other sources in dairy cattle farms. However, the difference in the prevalence of MRSA isolated from these two sample types was not significant. Moreover, MRSA transmission among cattle, humans, and the environment cannot be ruled out. Therefore, MRSA monitoring and prudent antimicrobial use in dairy cattle farms should be regularly implemented.

Regarding univariable and multivariable meta-regression, there was no significant association between the overall pooled prevalence of MRSA and any variable, suggesting that the source of heterogeneity could not be explained by the year of publication, continent, or sample type. It suggests that the heterogeneity of reported prevalence among included publications might be associated with other factors, such as the method of isolation, the sampling and sample handling procedure, and the history of MRSA infection or transmission in the farms. However, the information regarding those factors was not equally and well-explained in most of the included publications. Therefore, they were not extracted during the systematic review and included in the meta-analysis. A further study with a more specific hypothesis using different search strategies and inclusion and exclusion criteria should be performed to investigate the source of heterogeneity of the prevalence of MRSA in dairy cattle farms.

Analysis of publication bias performed using the funnel plot and Egger's test revealed the bias of publications with small-study effects. Small-study effects are generally defined as a phenomenon in which studies with smaller sample size show different, and often larger, effects than studies with a larger sample size. This phenomenon can be due to the publication bias, when small studies reporting larger effects are more likely to be published compared to those reporting smaller effects. A funnel plot, showing the reported effects from small studies which are usually associated with high standard errors and large studies which are usually associated with low standard errors, can be used to illustrate the publication bias. According to the present study, the funnel plot clearly shows that small studies reporting low prevalence are missing which is illustrated as an area without any dots in the bottom left corner of the plot. Even though the small-study effect is a potential limitation of this study, all included publications were of fair to high quality. Moreover, using a sensitivity analysis, we showed that our meta-analysis was robust and stable. Other study limitations should also be concerned. First, only articles that were written in English were included. Second, the included studies were obtained from only three distinct databases. Third, the year of publication of several studies was not identical to the year of MRSA isolation, which may have influenced the misclassification and misinterpretation of the subgroup meta-analysis. Even though we successfully revealed the global prevalence of MRSA in dairy cattle farms, other knowledge such as the risk factors associated with the presence of MRSA and the antimicrobial use in dairy cattle farms was not described. This gap in knowledge is critical in controlling and monitoring MRSA in dairy cattle farms and needed to be further investigated in a future study.

Conclusion

The global pooled prevalence of MRSA in dairy cattle farms has been minimal yet consistent over time. The pooled prevalence of MRSA in dairy cattle farms was the highest in Asia, followed by Africa and Europe. Cattle milk samples were found to harbor a higher prevalence of MRSA than other sample types. Therefore, following the results of this study, we recommend that appropriate levels of barn sanitation, personnel sanitation while handling animals and animal products, implementation of a continuous surveillance and monitoring program for evaluating animal health, and monitoring of antimicrobial resistance patterns at the farm level, be employed to control the spread of MRSA in dairy cattle farms.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

SB and NA designed this study. SB, SK, and NA performed the systematic review and meta-analysis. SB and SK prepared and revised the manuscript accordingly. All authors contributed to the article and approved the submitted version.

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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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2022.947154/full#supplementary-material

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Women's participation on the boards of farmer-owned cooperatives

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Initiatives and specific measures aimed at increasing the presence of women on corporate boards have become widespread. However, not much academic attention has been paid to this subject up till now, when it comes to farmer-owned cooperatives. The article shows that farmer-owned cooperatives do have special problems when it comes to women on boards. The farmer-owned cooperatives in Denmark have been chosen as cases in this article, as they are quite big, exposed to international competition and have substantial market power. Based on annual reports from 25 farmer-owned cooperatives and two of their investor-owned subsidiaries in the years 2005–2022, inputs from present and former board members of farmer-owned cooperatives, CSR-reports etc. a number of conclusions are drawn. Cooperatives have particular challenges with regard to gender diversity on boards due to their specific structure and requirements—compared to investor-owned companies. Different types of barriers that limit women's representation on boards can be identified: (1) Institutional barriers in terms of statutes and cooperative principles. (2) Structural barriers in the form of a narrow or skewed recruitment base. (3) Historical and cultural barriers, where agriculture is typically a male-dominated business. Women's representation on boards of farmer-owned cooperatives is relatively low but increasing. From 2005 to 2021 the weighted average share of female board members has increased from about 1–20%. Gender diversity in farmer-owned cooperatives is consistently less than in listed companies. The increasing representativeness of women is primarily due to the presence of more female external members. Since 2013 the proportion of women has increased, and in 2021 there were more female than male external board members. Female board members are more common in the large farmer-owned cooperatives than in the small. A positive correlation between the size of the companies and the representation of women is identified. This is supported by large cooperatives' greater focus in annual reports and CSR strategies on women's representativeness. Based on the cooperatives' diversity policy, their explicit and specific goals for women's representativeness on boards, interviews with board members etc. a clear awareness of the challenge of gender diversity on the boards is identified.

KEYWORDS

diversity, barriers, external board members, gender, recruitment pool, agriculture, representativeness

1. Introduction

1.1. The relevance of the topic

During the last decades, initiatives and specific measures aimed at increasing the presence of women on corporate boards have become widespread. Norway, in 2005, was the first country to pass a quota law generally requiring at least 40% women on the boards of listed companies (though somewhat dependent on the total number of board members). Subsequently, several other European countries, including Belgium, France, Germany, Iceland, Italy, the Netherlands, Portugal, and Spain have adopted their own board gender quotas. The quotas vary across countries, some with Norway's 40% requirement and others with less (1).

In June 2022, The European Parliament and the EU countries' negotiators finally agreed on a directive to increase the presence of women on corporate boards (2). The aims are to ensure gender parity on boards of publicly listed companies in the EU, and also to ensure that at least 40% of non-executive director posts or 33% of all director posts are occupied by the under-represented sex by 2026.

Small and medium-sized enterprises with fewer than 250 employees are excluded from the scope of the directive. Farmer-owned cooperatives are *per se* also excluded as they are not publicly listed companies. Farmer-owned cooperatives do, however, have special problems when it comes to women on boards:

- According to their statutes, only farmers have the right to become board members, and the overwhelming majority of farmers are men. This means that the recruitment pool for women is relatively small.
- For cultural and historical reasons, there is a tradition for men to have a dominant share of the board positions in farmer-owned cooperatives. This creates a barrier than might be difficult to change.
- The recruitment pool among cooperative owners is continuously shrinking as a consequence of the structural development in agriculture.
- Cooperatives have become larger and more international. The role and responsibilities of the board members—regardless of gender- have increased significantly, which means increasing demands on their specific and diverse skills.

Furthermore, not been much attention has been paid to this subject up till now and not a lot of literature on the topic exists, which may seem surprising given the importance of farmer-owned cooperatives but also the increasing focus on gender diversity.

Therefore we in this article wish to examine the following hypotheses:

- Cooperatives have special challenges with regard to gender diversity on the boards, due to their specific structure and requirements. Structural, cultural, historical, and institutional barriers limit women's representation on boards.
- Women's representation on boards of farmer-owned cooperatives is relatively low but increasing.
- The increase is primarily due to increases in the numbers of female external board members.
- There is a larger share of female board members in the large farmer-owned cooperatives than in the small.
- There is a clear awareness of the challenge of gender diversity on the boards.

The purpose is not to make normative assessments of women's participation in boards of farmer-owned cooperatives. The starting point for the article is that several stakeholders want more women in the top management (employed and elected) of cooperatives, and legislative initiatives also make this topic relevant. From a diversity point of view, the distribution of board members is skewed which, from a purely business perspective, is also a possible sign of inefficiency. The hypothesis above are analyzed based on observational cross-sectional as well as longitudinal data and a positivistic research philosophy.

Women's participation in cooperatives can take place through several forums: through the board of directors, board of representatives or general meeting, through committees and working groups set up by the cooperative or through ongoing discussion, exchange of ideas and constructive dialogue with the cooperative as shareholder. The concept of active ownership in cooperatives is widespread, and the intention is to activate all members in the discussion about the operation and development of the cooperative. Strategies for the cooperatives must be decided by the board of representatives, but the board—supported by the management—is typically the executing party and the central player when it comes to strategies and future development of farmer owned cooperatives. In this research we focus on women's participation on the boards of farmer-owned cooperatives, while women's participation in other forums is left for further research.

For several reasons, the conditions and the farmer-owned cooperatives in Denmark have been chosen as cases in this article: Farmer-owned cooperatives are rather common and important in the Danish agricultural and food industry, which is furthermore highly developed and exposed to international competition. Some of the farmer-owned cooperatives in Denmark are among the largest in the world within its business segment, demanding a high degree of competencies and leadership from the owners. Furthermore, information about board members of Danish companies is readily available.

It is, however, assumed that the identified problems, hypotheses and possible solutions are relatively generic and relevant for farmer cooperatives in most countries, perhaps especially the most economically developed ones.

1.2. Farmer-owned cooperatives: Uniqueness, relevance, characteristic etc.

This article focuses on farmer-owned cooperatives alone. Farmer-owned cooperatives have substantial differences compared to, for example, investor-owned companies. Especially in relation to women's participation on the boards, special conditions apply (as described below), which can constitute significant barriers, making it pertinent to focus on this selected business type.

There is no universally accepted definition of a cooperative. In general, a cooperative is a business owned and democratically controlled by the people who use its services and whose benefits are derived and distributed equitably on the basis of use. The user-owners are called members.

The International Cooperative Alliance (ICA), defines a cooperative as "an autonomous association of persons united voluntarily to meet their common economic, social, and cultural needs and aspirations through a jointly-owned and democratically-controlled enterprise" (3).

Another widely accepted definition is that "A cooperative is a user-owned, user-controlled business that distributes benefits on the basis of use" (4). This definition captures what are generally considered the three primary cooperative principles: User ownership, user control, and proportional distribution of benefits.

Farmer-owned cooperatives often base their regulations on the seven international cooperative principles established by the ICA:

- Voluntary and Open Membership.
- Democratic Member Control.
- Member Economic Participation.
- Autonomy and Independence.
- Education, Training and Information.
- Co-operation among Co-operatives.
- Concern for Community.

Several of these principles are important when it comes to women's participation on the boards of farmer-owned cooperatives.

Cooperatives are democratic organizations controlled by their members, who actively participate in setting their policies and making decisions. This usually means that the members themselves personally join the boards. As a consequence, the farmers themselves enter the board of farmer- cooperatives, which significantly limits the diversity of the board's recruitment pool.

The limited diversity is further reinforced by the fact that the number of farmers, and thus the number of directly eligible members of the cooperatives' boards, decrease over time. As shown in Figure 1, the number of farms in Denmark has decreased by almost 50% since 2000, and especially the number of full-time farms has decreased.

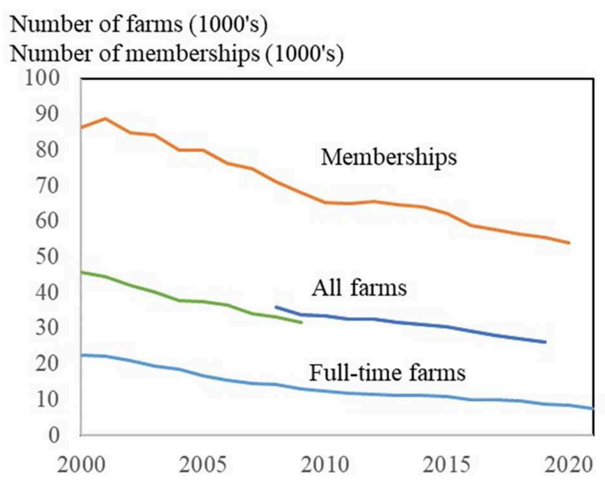

FIGURE 1
Development of farms and farmer-cooperative memberships in Denmark. All farms: Discontinued time series 2008–09. Source: Own calculations based on Statistics Denmark (5) and annual reports from companies and organizations.

During the period of 2000–2020, the number of memberships of farmer-owned cooperatives has also decreased significantly. It is worth noting that the number of memberships is greater than the number of farms. The explanation is that a farmer is typically a member of several cooperatives.

Cooperatives are by definition autonomous, self-help organizations controlled by their members. Members are thus numerous and unconcentrated, as all members have equal voting rights (one member and one vote).

However, the cooperative may also choose board members outside the ownership group. This is typically the case if individuals with special leadership competences etc. are required. In these cases, which are becoming more and more common, access to professional competences, experience and, not least, complementarity are important characteristics.

Unlike cooperatives, the owners in investor-owned companies have influence in the sense of one dollar, one vote. Voting rights depend on the amount of money invested *via* the number of shares. Investors can appoint other people to the boards. As long as they comply with specific competence requirements and are fit-and-proper, there are few restrictions when it comes to appointing people to participate in board work, i.e., regarding nationality, gender, etc. Therefore, the pool for recruitment is far broader than in cooperatives. Conversely, the board members of a cooperative often have a very keen interest in the company, and have strong personal (economic) incentives for ensuring a profitable and long term development of the company.

Overall, the conclusion for now is that cooperatives and investor-owned companies of course have some similarities but also clear differences. Both types of companies develop over time and thus it is a recurrent feature that modern cooperatives, in many cases, have developed so that they share many features with investor-owned companies. Generally, there has been a tendency for cooperatives to increasingly focus on business, while non-economic, non-commercial and ideological aspects have become less important. In addition, several hybrid models have developed which are crosses between cooperatives and investor-owned companies, or which contain both cooperatives and investor-owned companies in the same company.

Cooperatives and investor-owned companies are therefore far from unambiguous company structures and there can be large differences between different types of cooperatives, and also big differences from country to country. Despite this, it is possible to identify a number of general and important similarities and differences between cooperatives and investor-owned companies (6).

The prevalence of farmer-owned cooperatives varies considerably from sector to sector and from country to country, which can partly be explained by the different market conditions, which to a greater or lesser extent stimulate the need for—and the benefits of—the cooperative organization. Specifically for cooperatives owned by farmers, it is evident that cooperatives are most widespread in North America, Northern and Central Europe, as well as in Japan and Korea.

In general, cooperatives, regardless of sector and industry, are most important in the most economically developed countries. Cooperatives in these countries have a relatively large market share and many farmers are typically members of one or more cooperatives. An important explanation for this different importance between countries is that the establishment and management of cooperatives requires a certain level of infrastructure, training and organization, which is not always present in the less developed countries.

When it comes to differences between industries, the cooperative organization is particularly common in the supply and processing activities that are closely linked to agricultural production in the value chain, i.e., downstream, whereas farmer-owned cooperatives are rarely found in activities close to consumers. This producer- rather than consumer-orientation may also be a factor in women's representation on boards of farmer-owned cooperatives.

1.3. Farmer-owned cooperatives in Denmark: Management challenges

The purpose of this section is to illustrate and document that the farmer-owned cooperatives in Denmark are quite big and exposed to international competition, which in turn means that management, tasks and responsibilities are quite demanding and business oriented.

That the Danish farmer-owned cooperatives have substantial market power and faces international competition is evident from Table 1 below, which shows the market shares for farmer-owned cooperatives in Denmark in the major agri-industrial industries.

TABLE 1 Farmer-owned cooperatives' shares of their respective markets in Denmark in 2021 (or latest year with available data).

Product	Percent
Whole milk deliveries	92
Butter	99
Cheese	92
Pork	72
Beef	63
Grass seed	76
Egg	40
Sugar	0
Poultry	0
Agr. machinery	0
Fruit and vegetables	53
Feed and fertilizer	80
Potato starch	100

Pork and beef: Share of slaughtering.
Eggs: The major shareholding egg company owned by farmer cooperatives is not included.
Fruit and vegetables: Fresh products for direct consumption.
Feed and fertilizer: Companies producing feed and selling fertilizer.
Source: Own calculations based on Hansen (7), Danish Agriculture and Food Council (8–10), and annual reports from companies and organizations.

Table 1 shows large market shares for farmer-owned cooperatives within the dairy and meat industry. The grass seed and the potato starch industries have gained a very strong competitive position, where the cooperative structure has been an important competitive strength (11).[1]

Contrary to this, the cooperative model has failed within the sugar, poultry and agricultural machinery industries. Cooperatives did exist in these industries, but for different reasons they could not compete, they failed or became unnecessary, and the cooperatives were subsequently acquired by other investor-owned or foreign companies.

The very different market shares for cooperatives from industry to industry can also be explained by the fact that the cooperative ownership has both advantages and disadvantages, which can have varying importance depending on structure, value chain, internationalization, capital intensity, etc. For example, cooperatives are common in the dairy sector around the world. The explanation is, that farmers who have daily production of fresh and perishable agricultural products, have a great incentive to secure right of delivery and a stable buyer (12).

1 Personal message, September, 2022, from Christian Høegh-Andersen, Chairman of DLF amba and DLF Seeds A/S.

Cooperatives have existed in Denmark since the 1880's. Cooperatives initially arose in the dairy and meat industry and subsequently spread to other agricultural branches (6). As illustrated in Figure 2, the cooperative market shares within Denmark have generally been stable in the recent decades, and growth has been achieved through foreign activities. This shows that the cooperative model has been viable and competitive in a period characterized by liberalization, globalization, and consolidation.

The increasing market shares for the feed and fertilizer industry is mainly due to acquisitions of investor-owned competing companies. To a certain extent, the acquisitions were a result of poor management of the acquired investor-owned companies, which was an important factor in why they were sold. This supports the fact that management is an important competitive parameter in these industries as well.

For many decades, most agricultural industries in Denmark have been strongly export-oriented—typically with export shares of 70–90%. More recently, growth has increasingly taken place through investments, production and establishment in foreign countries. This form of internationalization requires additional strategic business management. Internationalization through foreign direct investments, global strategic alliances and global mergers, which have been a massive trend in recent decades, are often difficult for cooperatives to cope with due to their structure and form of ownership: When the overall goal is to ensure the owners—the farmers—attractive sales prices and/or lower input prices, investments in foreign companies, sourcing of agricultural products from foreign farmers etc. can be perceived as irrelevant or aimless for the owners.

All in all, the Danish farmer-owned cooperatives are large and to a considerable extent engaged in international activities, as is illustrated in Table 2.

Table 2 shows selected key figures for the six largest farmer-owned cooperatives in Denmark. The respective estimates for the shares of foreign activities are also noted. Most of these companies are among the largest in Europe, or in the world, within their specific business area. This emphasizes that the needs for strategic management, and thus the requirements for the board members, are high.

1.4. Literature review

Gender diversity and women's representation in farmer-owned cooperatives is not a heavily analyzed topic. Topics such as gender diversity in business, diversity on corporate boards, management challenges in farmer-owned cooperatives etc. are well-analyzed, whereas the specific combination of the topics is much less studied.

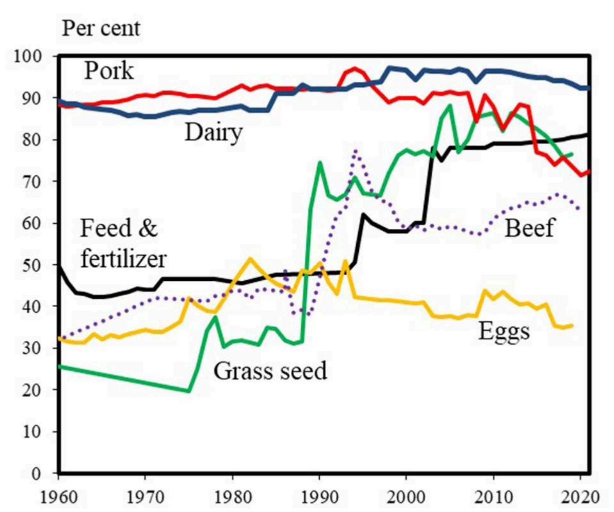

FIGURE 2
Domestic market shares for agricultural cooperatives in selected industries. Source: Own calculations based on Danish Agriculture and Food Council (8–10) and annual reports from companies and organizations.

Phil Kenkel studies board diversity in agricultural cooperatives, and underlines, that data on the board composition in agricultural cooperatives is limited (13). However, data from United States shows, that females make up just over 3% of board members in agricultural cooperatives—the lowest representation of any cooperative sector. It is concluded, that agricultural cooperatives clearly trail other cooperative sectors, as well as investor-owned firms, when it comes to board gender diversity.

Kenkel also touches upon the fact, that an increasing number of cooperatives are implementing an associate board structure as a way to increase diversity (13).

Aazami et al. analyze women's level of participation and the factors influencing their involvement in different stages of cooperative activities in Shiraz, Iran (14). The study concerns a women's cooperative and does not relate specifically to women's representation in farmer-owned cooperatives or to gender diversity. The analysis concludes, among other things, that women's participation was mostly at the level of "surrender" or "acceptance."

Women's active participation in cooperatives—as ordinary members or as members of the board, respectively—has some common barriers and limitations. A formal election to a board, including support from members, possible election campaign, etc., involves greater cultural and human barriers. Further, measures of gender representation typically relate to the board and not to general participation.

Food and Agriculture Organization of the United Nations (FAO) investigates agricultural cooperatives and gender equality, and notes that rural women have less access to the resources and opportunities in agriculture than men (15). FAO also recommends governments and international organizations to implement policies that foresee quotas or targets for women's participation in cooperative boards.

International Labor Organization (ILO) describes a survey about the relationships between the cooperative movement in general, women's empowerment and gender equality (16). Fifty percent of the survey respondents were from Europe, 15% from Asia, and 15% from North America. The study concludes, that "women are among the most involved in and served by

TABLE 2 Top-6 Danish farmer-owned cooperatives: Turnover and foreign activities.

		Turnover	Share of foreign activities	
Company	Business	Euro bn	Percent	
Arla Foods amba	Dairy	11.2	64	Share of milk intake outside home country
DLG amba	Farm supply	7.9	71	Turnover from foreign activities
Danish Crown amba	Meat	7.8	63	Employment abroad
Danish Agro amba	Farm supply	5.9	60	Turnover from foreign activities
DLF seeds	Grass seed	1.0	60	Assets in foreign countries
KMC amba	Potato starch	0.3	94	Export share

Amba means "cooperative with limited responsibility".
Arla Foods is a transnational cooperative with members in seven different countries but has its headquarter in Denmark and is formally registered in Denmark.
Source: Own calculations based on annual reports of the companies.

co-operative organizations, but among the least likely to hold high-ranking and decision-making roles" (16). When it comes to agriculture, the survey results suggest that women's participation in leadership in the agricultural sector in all regions of the world is significantly below the average for other sectors.

2. Materials and methods

The following five types of material and data have been considered for this article:

- Annual reports from 25 farmer-owned cooperatives (see Appendix) and two of their investor-owned subsidiaries in the years 2005–2022 (around 450 annual reports).
    - Purpose: Access to information about board members (number of members, member groups, and gender).
- Homepages from farmer-owned cooperatives.
    - Purpose: Access to information about board members (member group and gender) in present boards not yet publishing annual reports.
- CSR reports from farmer-owned cooperatives when available.
    - Purpose: Access to information about targets and goals for gender diversity.
- Relevant literature about or related to the topic of this article.
    - Purpose: To uncover existing literature in order to compare issues and solutions with other studies.
- Comments, statements, assessments from present and former board members of farmer-owned cooperatives.
    - Purpose: To have access to deeper information about barriers, drivers, motivations and attitudes regarding female participation in farmer-owned cooperatives.

Whilst the data primarily consists of annual reports from farmer-owned cooperatives, it is important to note that in two special cases data from investor-owned subsidiaries is also included. Since this impacts the subsequent results, this choice is explained and justified as follows.

In the subsequent analysis of gender diversity in farmer-owned cooperatives, the diversity is estimated both in a group consisting exclusively of cooperatives, and in a group where cooperatives' farmer-owned subsidiaries replace the parent cooperative. This is because a few large cooperatives have established investor-owned subsidiaries during the study period, which are wholly or almost wholly owned by the cooperative. This company construction is typically established for two reasons: First, by setting up an investor-owned subsidiary, the company is prepared for a situation where it is desirable to attract external investors as shareholders. Secondly, the purpose may be to make room for external board members and thus new management competence in the company. Often, the statutes of the cooperative will be a barrier to both external capital and external board members, which is why an investor-owned subsidiary can be an appropriate solution.

The model with the establishment of subsidiaries is only used where the most important strategic management takes place in the investor-owned subsidiary and where the cooperative is a majority shareholder. In this article, this is the case for two companies: DLF amba and Danish Crown amba.

DLF amba is a seed cooperative dealing in forage and amenity seeds, and other crops. DLF amba has for a long time almost fully owned the subsidiary DLF A/S, which is a limited company. Until 2021, DLF amba only had cooperative members (farmers) on the board, while DLF A/S, on the other hand, had a long tradition of having external members. From 2017, there was also a female external member of the board of DLF A/S. From 2021, the structure has changed, so that the board of DLF amba has been expanded and now also has external members, while the board of DLF A/S has been reduced and now only has cooperative members on the board.

Considering the dominant ownership of the cooperative in the subsidiary, we here consider the members of the boards of the limited subsidiary until 2020 and of the cooperative from 2021. In parallel with this, the members of the board of directors in the cooperative throughout the period are also included in the estimations.

Danish Crown amba is a food manufacturing cooperative in Denmark, dealing primarily in meat processing of pork and beef. It is Europe's largest pork producer. In 2006, the cooperative introduced two external board members, but in 2013 they were transferred to the 100% owned subsidiary, Danish Crown A/S, which is an investor-owned company. Since then, the number has increased to 4, and in 2022 50% of the board members are women. In the cooperative parent company, Danish Crown amba, only cooperative members are elected to the board.

We here count the members of the boards partly in the cooperative, partly in the cooperative's subsidiary with external board members depending on where the most important strategic decisions are made.

The cooperative dairy company, Arla Foods amba, is a special case: In 2019 the company appointed two external board members, in the beginning without the right to vote. From 2022, they have become full members with full voting rights. So whilst they do not have investor-owned subsidiaries, Arla Foods amba has still allowed for external members on the board. Throughout the period, only female external members have been appointed. We here include the external members in the estimations for the entire period, regardless of voting rights or not.

Regarding the choice of method, the empirical basis is not sufficient to carry out econometric or statistical analysis. The analyses will therefore be based on the collection and processing of primary data, descriptive statistics, as well as qualitative studies, which is considered to be the optimal method for investigating the topics of this article.

3. Results

Based on the material and the methods described in the previous section, a number of interesting results have been obtained, which can be used to confirm or reject the established hypotheses.

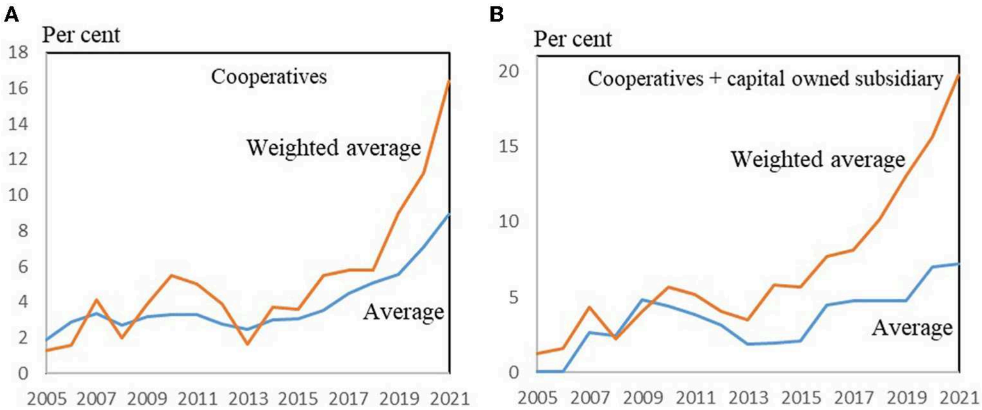

FIGURE 3
Female board members (share of all board members) of Danish farmer-owned cooperatives, 2005–2021. **(A)** Average of 25 farmer-owned cooperatives. **(B)** Cooperatives + cooperatives with a shareholding subsidiary with external members. Source: Own data collection, calculations and presentation based on annual reports and homepages of 25 farmer-owned cooperatives in Denmark.

In Figure 3, the overall trend for female representation on the boards since 2005 are shown for both the cooperatives (A) and for the cooperatives + investor-owned subsidiaries (B).

In Figure 3, the "Average" means a simple average of the number of female board members as a percentage of all board members in the 25 farmer-owned cooperatives. For the "Weighted average" the share is weighted by the size of the companies as indicated by the turnover. The 25 cooperatives are of very different sizes, ranging from 20 million Euros to 10 billion Euros in annual turnover in normal years.

In Figure 3 the trend for female representation on the boards since 2005 is relatively clear, regardless of whether only cooperatives (A), or cooperatives + investor-owned subsidiaries (B) are considered: women's shares on the boards of cooperative agricultural companies is increasing, and especially in the last 5–10 years a significant increase can be seen.

The boards under B are only considered in cases where the most important strategic management takes place in the investor-owned subsidiary and where the cooperative is a significant majority shareholder (c.f. Section 3 above). Women's average representation on the boards is larger when these subsidiaries are also considered, as can be seen when comparing the left and the right panel of Figure 3.

Figure 3 also shows that the weighted shares are considerably larger than the unweighted shares, and that the differences are increasing. It shows that women's representation is larger in large companies. The increase in women's representation has mainly occurred in large companies.

To investigate this pattern further, Figure 4 illustrates the relationship between the size of the companies and the share of women on the boards of the individual companies.

Figure 4 shows a positive correlation between the size of the companies and the representation of women, where we see that the largest cooperatives have the largest shares of women on their boards (15–25%). The small companies are more varied, with between 0 and 23% women on their boards. The correlation is also supported by the greater focus on diversity in the large cooperatives' annual reports. Note that we do not here argue causality, nor hypothesize on the direction of any possible causality between size and female representation, as any casual relationships between board composition and companies' performance are usually difficult to verify.

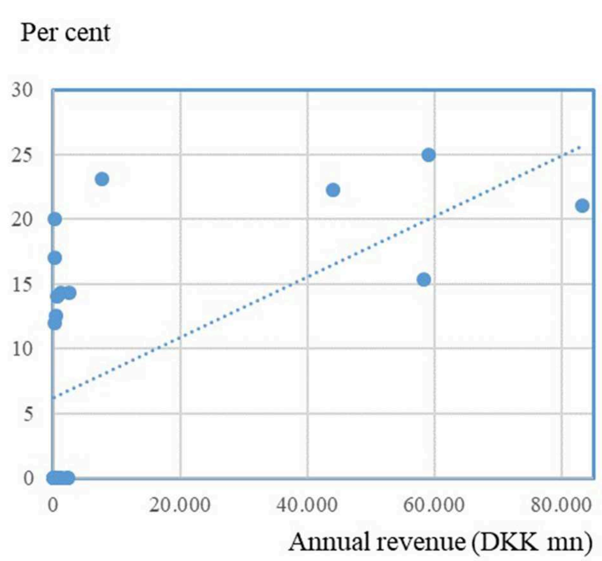

FIGURE 4
Size of farmer-owned cooperatives and share of women on the boards. Source: Own data collection, calculations and presentation based on annual reports and homepages of 25 farmer-owned cooperatives in Denmark.

Specifically regarding female board members and based on Norway's relatively long-term experience, Eckbo et al. conclude, that mandatory board gender-balancing did not reduce firm value or performance significantly (1). Another study concludes, that the average effect of gender diversity on firm performance is negative (17).

Board membership for the farmer-owned cooperatives takes place in three membership categories: Cooperative members (farmers), employees, and external members.

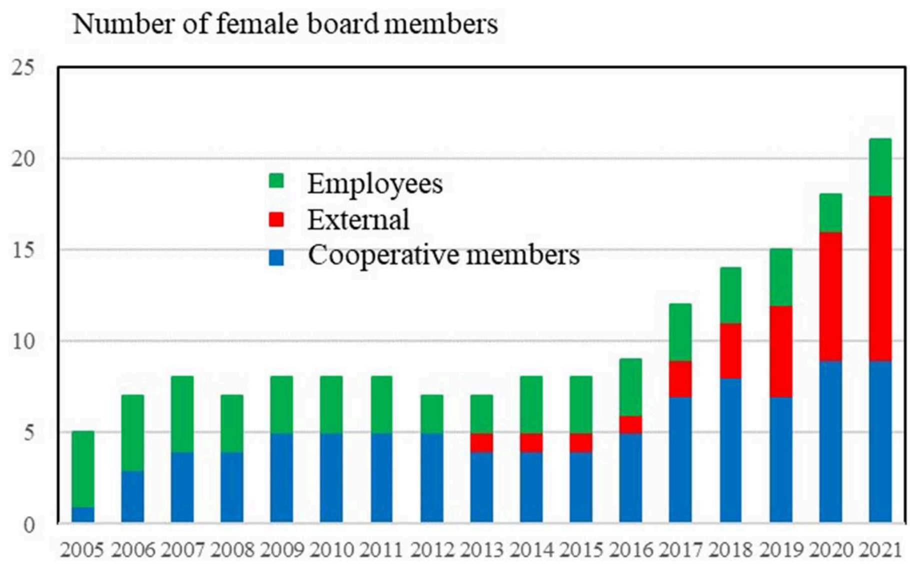

FIGURE 5
Groups of female board members of Danish farmer-owned cooperatives, 2005–2021. Source: Own data collection, calculations and presentation based on annual reports and homepages of 25 farmers owned cooperatives in Denmark.

Cooperative members are elected in accordance with the statutes among the active members of the cooperative. Typically farmers, or managers on larger farms, can vote and can be elected. Each farmer or farm has one vote.

The employees also have the right to be represented on the boards according to the present legislation, however this depends on the company's size and form of ownership. In companies with at least 35 employees, the employees have the right to elect a number of board members corresponding to half of the other members (18).

Finally, several cooperatives have now introduced a third group of members, external members. In these cases, the statutes allow the general meeting and/or the board members to give external persons a seat on the board.

The starting point is that all board members have the same influence and responsibility, so it is reasonable to consider all members of the board as one, regardless of member group. This is the assumption when assessing women's influence and representativeness. It should be noted, however, that cooperatives and their management have no influence on whom the employees choose for the boards.

Figure 5 shows the total number of women in farmer-owned cooperatives 2005–2021 for each member group.

Figure 5 shows that the increasing representativeness of women is primarily due to the presence of more female external members. Women first appeared as external board members for the agricultural cooperatives in 2013, and since then the number has increased to around 43% of all women on the boards in 2021. The number of female cooperative members on the boards is increasing, but at a slower pace and from a relatively low level. Approximately 9% of cooperative board members in 2021 were women.

Among the employees, women's representation has been rather stable, and they make up a relatively small proportion of the total number of women on the boards (14% in 2021). In contrast, female external members are now as large a group as female cooperative members.

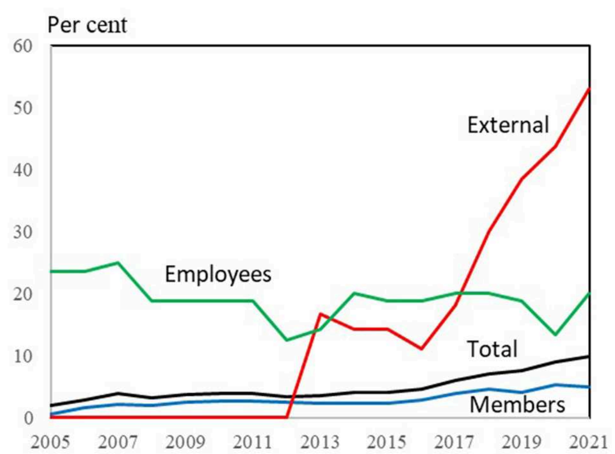

FIGURE 6
Board member groups of Danish farmer-owned cooperatives, 2005–2021: Share of female board members. Source: Own data collection, calculations and presentation based on annual reports and homepages of 25 farmers owned cooperatives in Denmark.

Women's increasing representation *via* the role of external members can be seen as a result of the cooperatives' initiatives to increase women's representation within existing statutes: With a small recruitment pool, it is difficult to increase the proportion of female cooperative members on the boards. Given these limited options, the appointment of female external members is a relatively easy way to increase women's representation on boards.

The development in women's shares of board members in the various member groups is also illustrated in Figure 6.

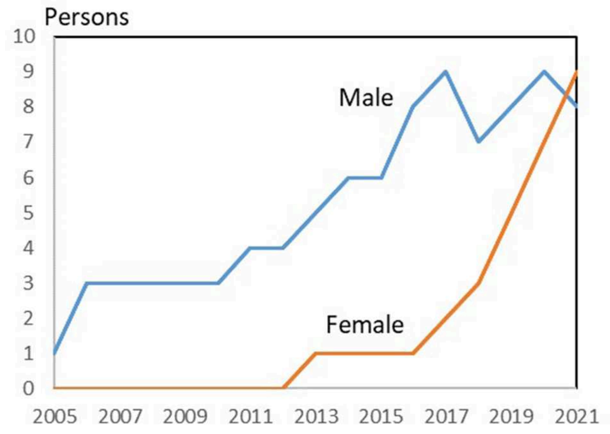

FIGURE 7
External members of Danish farmer-owned cooperatives: Male and females. Source: Own data collection and presentation based on annual reports and homepages of 25 farmers owned cooperatives in Denmark.

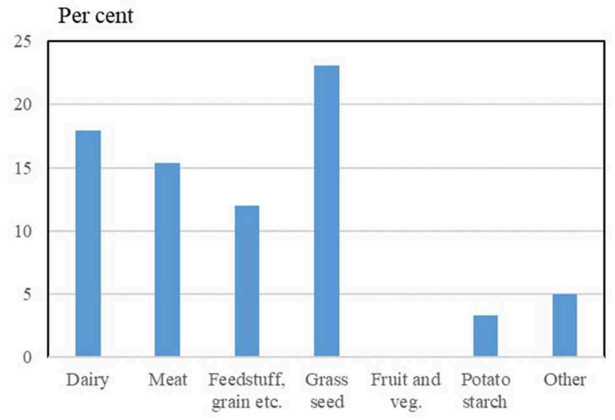

FIGURE 8
Share of women on boards of farmer-owned cooperatives in different sectors (2021). "Other" includes primarily fur-feed cooperatives and an egg wholesale cooperative. Source: Own data collection and presentation based on annual reports and homepages of 25 farmers owned cooperatives in Denmark.

Figure 6 shows both the level and the development of women's representation in the various member groups. The employees contribute to a higher total share throughout the period, while external members contribute a lot in the last part of the period. In 2021, women made up only 5% of the member-elected board members.

The increasing representativeness of women *via* external members is evident from Figure 7, which shows the male and female share of external board members of Danish farmer-owned cooperatives.

The figure shows that male external board members have existed throughout the period, while women have only been appointed as external members since 2013. Since then, the proportion of women has increased, and in 2021 there, for the first time, were more female than male external board members.

Women's representation on cooperative boards probably varies from sector to sector. Some sectors may be more or less male-dominated based on e.g., historical reasons. The differences in women's shares of board positions in different sectors are shown in Figure 8.

However, the differences between the sectors must be interpreted with caution, as the structure in the individual sectors is very different. The sector "meat industry" thus consists of only one large company, while the group other "industries" consists of many but relatively small companies.

Companies, regardless of ownership but of a certain size, are obliged to set targets and present policies for the underrepresented gender, and report on this. Several cooperatives thus have more or less explicit and concrete targets for gender diversity on the boards. It ranges from rather vague intentions to specific goals with both numbers and times specified. Concrete action plans can also be included in companies' diversity policy in their CSR reports.

Explicit goals or targets for women's representation on the boards of the cooperatives for the whole farmer-owned cooperative industry are, however, difficult to quantify when the degree of specificity is so different, has different time horizons, and sometimes is completely non-existent. Based on data from the cooperatives with rather explicit goals for gender diversity, the potential for women's further representativeness on boards can be calculated, and are illustrated in Figure 9.

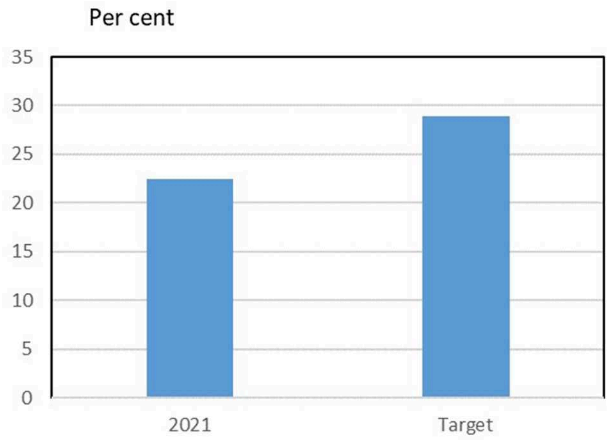

FIGURE 9
Female board members (present and target) of Danish farmer-owned cooperatives. Includes five farmer-owned cooperatives with explicit and quantitative target for share of female board members. Targets refer to cooperative members, and external and employee members are assumed to be unchanged. Targets are expected to be met in different years in the period 2004–2030. Source: Own data collection and presentation based on CSR-reports and homepages of farmers owned cooperatives in Denmark.

Companies' goals, ambitions and intentions regarding gender diversity cannot be used uncritically as an estimate for future development. However, the figure shows that the targets are above the current level. Still, the goals do not reflect full gender equality, so even if the goals are met, women will be underrepresented. However, the goals can be said to be realistic and not an expression of a long-term ambition.

4. Discussion

The analysis and the results found in the previous chapters have led to new issues, questions, discussions and perspectives arising. Several barriers have been identified and revealed, and the recruitment pool for cooperatives seems to be a consistent theme, and the problems with the recruitment of more women on the boards may be almost chronic and intractable for the cooperatives in several ways:

- The number of cooperative members is decreases continuously, and thus the recruitment basis among members becomes smaller. The recruitment pool among potential female board members will therefore also be smaller, which—ceteris paribus—makes the companies more vulnerable.
- Women already make up a very small proportion of the members of the cooperatives.
- Historical, institutional, and cultural barriers seem to limit women's share of the recruitment pool in agriculture. The persistent low number of women in agriculture and limited opportunities for external members according to statutes make it difficult to increase gender diversity significantly.
- The statutes and the business ideas of farmer-owned cooperatives idea are based on the premise that farmers participate actively and elect board members among themselves. The options for female external board members exist, and are used, but have limited potential. External board members should not have a dominant role in the boards, if the principles in cooperatives are to be followed. The farmers and the members will probably also be very reluctant to hand over the majority influence—or just a significant influence—on the boards to external members, cf. for example an interview with a former president of the Danish Agriculture Council, Peter Gæmelke (19).

The rather modest recruitment pool is a difficult barrier. The question is whether the barriers are structural and long-term, and whether they are expected to continue.

In Denmark, on which this study is based, female farmers own only 5% of the agricultural land, while men own 81%, and the rest is owned by companies (20). The share has been rather constant between 2010 and 2019 (21). Approximately 20% of the employees in agriculture, forestry and fishing together are women (21).

Men also make up the dominant proportion of members of cooperatives, of their representatives, and of the delegate assembly (Table 3).

Definitely, the recruitment pool of potential female board members among farmers is modest, and it will stay low for a long period due to structural, traditional and historical reasons, since the number of farmers and farms is continuously decreasing, and women consistently constitute a very small proportion of them. These structural conditions seem to be quite persistent.

The chairman of a farmer-owned cooperative with a very low number of female members, Karup Kartoffelmelsfabrik (AKK amba) himself considers that a certain gender diversity in a cooperative's board and management is to the advantage of the company, ceteris paribus. This is also something the company both strategically and specifically works for. However, the recruitment pool is a significant barrier. As an example, typically a maximum of five out of ∼125 participants at the general meeting in Karup Kartoffelmelsfabrik are women, and in that forum members to the board of directors are elected. This corresponds to 4%, compared to the current proportion of women on the board of 14%.[2] As shown in the Table 3, <2% of the farmers or members are women.

TABLE 3 Women's shares (percent) of members in farmer-owned cooperatives (2022 or recent years).

Cooperative	Percent	Recruitment pool
Arla Foods amba	14	Farmer-elected members of the board of representatives
Danish Crown amba	3	Farmer-elected members of the board of representatives
DLG amba	15	Farmer-elected members of the board of representatives
Danish Agro amba	10	Delegate assembly
DLF amba	2	Delegate assembly
AKK amba	<2	Members
AKM amba	4.5	Members
AKS amba	1.6	Members

Source: Own presentation based on CSR-reports from the individual cooperatives.

Other board members in farmer-owned cooperatives also indicate the small recruitment base as one of the most important barriers to increased gender diversity on their boards. According to a farmer with long-term experience as a board member and chairman in a number of both farmer owned cooperatives and investor owned companies, the relatively small share of women in agriculture is a significant institutional explanation. However, other factors are also important: It turns out that once women constitute a certain proportion of the elected members, it becomes easier to attract additional women to the boards. Legitimacy increases the more women are elected. Several other cultural barriers also exist, but a focused and persistent effort can reduce them.[3]

Culture and traditions are also highlighted as specific barriers for women on boards. For example, the chairman of Arla Foods emphasizes that it is a question of culture when less women are represented at the top of the cooperatives in Denmark than in e.g., neighboring Sweden (22). He points out that influencing and changing the culture in agriculture in order to improve gender diversity is probably a very difficult challenge.

The specific initiatives to attract more women to the boards vary greatly in form and scope from cooperative to cooperative. An example is DLG amba, which in recent years has increased the number of both external and member-elected female board members. Since 2019, DLG has thus increased the proportion of

2 Personal message, August 5, 2022, from Kristian Møller Sørensen, Chairman of Karup Kartoffelmelsfabrik amba and board member of KMC amba.

3 Personal message, December 15, 2022, from Niels Dengsø Jensen, Chairman of DLG amba (Danish Farm Supply Cooperative), chairman of AP Pension, DanHatch Holding, Vilofoss etc.

women on the board (cooperative members and external members) from 0 to 23%. As a specific measure to achieve the goals of greater female representation, DLG had advertisements in the agricultural media and held an inspiration meeting (23).

Arla Food, which has also increased the representation of women on the board in recent years, has no concrete initiatives to attract more member-elected women to the board. According to the chairman, the ambition to have more women on the board of representatives is achieved by formulating and explaining the issue in the relevant fora (22).

Initiatives such as special quotas have also been discussed in the cooperatives. The chairman of DLF amba emphasizes that he would like to see more women on the board, but no plans for quotas or other active measures to get more women on the board have been suggested or implemented. The reason is that the members are elected in constituencies where they elect the delegates. It is a democratic system that works as intended, and qualifications are given the highest weight (24). It shows a potential conflict between the perception of democracy and diversity.

As has been shown previously, in recent years gender diversity on average in Danish farmer-owned cooperatives has improved substantially. The questions are whether a reasonable level of diversity has now been ensured, and whether an independent gender diversity problem in farmer-owned cooperatives exists. However, the average figures cover a considerable spread: The figures for the individual companies show, that half of the cooperatives in this survey have no women on the boards at all.

Several large cooperatives have presented diversity goals which show that there is still a need for more women on the boards in order to achieve the goals, some of which are even quite short-term.

Even with the presented diversity goals, there is a long way to full gender diversity. In reality, coming close to something resembling full equality, which may not be a target, is probably impossible, as the share of external board members hardly can be increased substantially any more, as statutes prevent new access. Furthermore due to the cultural and historical barriers, the number of member-elected women in the boards is likely to be relatively permanent.

Compared to other industry sectors, it appears that farmer-owned cooperatives have—or have had—a gender diversity problem of their own. As Figure 10 shows, gender diversity in farmer-owned cooperatives is consistently less than in listed companies.

Figure 10 shows a general and significant increase in share for all four groups of companies. The five largest farmer-owned cooperatives, which most of all can be compared to listed companies, have since 2015 increased the representation of women on the boards so much that they are now almost at the level of listed companies. For all farmer-owned cooperatives on average—and thus in particular for the smaller farmer-owned cooperatives—the diversity gap is still significant.

The increased proportion of women on the boards of farmer-owned cooperatives is primarily, and almost exclusively, due to more external female board members, i.e., non-members and thus non-farmers. Typically, the purpose has been to supplement the boards with competencies that did not exist to a sufficient extent among the member-elected board members. An interesting characteristic is the fact that the proportion of women is far greater among the external members than among the member-elected members. However, in a democratically led company, it is also important that the owners are well-represented and that they have the necessary influence to ensure that the company develops according to the purpose. A balance must be ensured: On the one hand, the board must be anchored among the owners to ensure both legitimacy and support. On the other hand, external female board members can ensure both better gender diversity and access to necessary management competencies on the board.

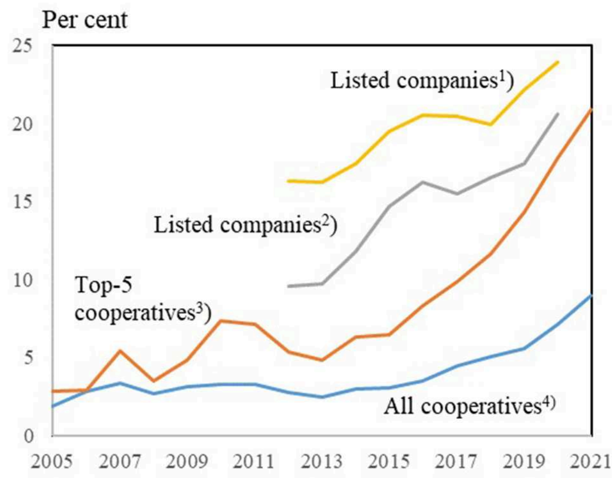

FIGURE 10
Women's shares (percent) of all board members in different types of companies in Denmark. [1]Elected by the general meeting and by employees in listed companies. [2]Elected by the general meeting. [3]Elected (total) in farmer-owned Danish farmer-cooperatives: Five biggest cooperatives. [4]Elected (total) in farmer-owned Danish farmer-cooperatives: All cooperatives. Sources: Own calculation and presentation based on (25) and annual reports from cooperatives.

5. Conclusion

Although the discussion identifies or exposes new barriers and issues, the hypotheses set out at the beginning of this article can all be confirmed based on the analyses, results, and discussions.

Cooperatives have particular challenges with regard to gender diversity on boards due to their specific structure and requirements, compared to investor-owned companies. Different types of barriers that limit women's representation on boards can be identified:

- Institutional barriers in terms of statutes and cooperative principles.
- Structural barriers in the form of a narrow or skewed recruitment base.
- Historical and cultural barriers, where agriculture is typically a male-dominated business.

Women's representation on boards of farmer-owned cooperatives is relatively low but increasing. From 2005 to 2021 the weighted average share of female board members has increased from about 1 to 20%. Gender diversity in farmer-owned cooperatives is consistently less than in listed companies.

The increasing representativeness of women is primarily due to the presence of more female external members. Women have only been appointed as external members since 2013. Since then, the proportion of women has increased, and in 2021 there were more female than male external board members.

Female board members are more common in the large farmer-owned cooperatives than in the small. A positive correlation between the size of the companies and the representation of women is identified. This is supported by large cooperatives' greater focus in annual reports and CSR strategies on women's representativeness.

Based on the cooperatives' diversity policy, their explicit and specific goals for women's representativeness on boards, interviews with board members etc. a clear awareness of the challenge of gender diversity on the boards is identified.

The article both uncovers and quantifies the problem—also in a time perspective in a case which, from a farmer-cooperative aspect, is relevant, although not fully representative in an international perspective. Such an analysis of this size has not been carried out before, and both the visibility of the problem and the mismatch between goal and result is useful for stakeholders in and around farmer-owned cooperatives.

When it comes to theoretical implications, the structural and institutional problems uncovered in the article are significant barriers to women's participation in farmer-owned cooperatives. New or alternative models and solutions must be presented and assessed. This applies, for example, to external members of the boards, who to a certain extent can solve the problems, but who also create new problems.

Specifically regarding managerial implications, challenges and unresolved issues, which necessitate managerial considerations, are identified. Explicit goals, instruments and time horizons regarding gender representation are explicitly missing in several cooperatives, which *per se* is considered to be a problem. Recognition of generally accepted goals about gender representation is an important managerial achievement. A gap between goals and results is in itself also a managerial challenge that should be solved in the short or longer term. The managerial task consists of motivating women, making board membership attractive and, not least, showing and documenting the benefits of more women on boards.

As to future research three topics deserve a deeper analysis:

First, this article is based on a case study. In order to obtain more representative conclusions, it is relevant to study other countries as well: Are the issues, goals, initiatives and results different, and what conclusions and recommendations can be drawn?

Secondly, the topic lacks more specific analyses of goals or ambitions vs. real observations for women's participation: Do farmer-owned cooperatives have goals for women's participation, are they in line with general recommendations, and how big is the gap between goal and result?

Thirdly, qualitative and quantitative studies regarding the impacts of women's participation will be both relevant and useful. It concerns both the work and management of the boards and potential impacts on the performance of the cooperative.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

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

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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Appendix

Names of and links to farmer-owned cooperatives in Denmark

Leverandørselskabet Danish Crown amba (CVR: 21643939).
https://datacvr.virk.dk/enhed/virksomhed/21643939?fritekst=21643939&sideIndex=0&size=10
Danish Crown A/S (CVR: 26121264).
https://datacvr.virk.dk/enhed/virksomhed/26121264?fritekst=26121264&sideIndex=0&size=10
Arla Foods amba (CVR: 25313763).
https://datacvr.virk.dk/enhed/virksomhed/25313763?fritekst=25313763&sideIndex=0&size=10
DLG amba—Dansk Landbrugs Grovvareselskab (CVR: 24246930).
https://datacvr.virk.dk/enhed/virksomhed/24246930?fritekst=24246930&sideIndex=0&size=10
Danish Agro (CVR: 59789317).
https://datacvr.virk.dk/enhed/virksomhed/59789317?fritekst=59789317&sideIndex=0&size=10
DLF amba—Dansk Landbrugs Frøselskab (69459218).
https://datacvr.virk.dk/enhed/virksomhed/69459218?fritekst=69459218&sideIndex=0&size=10
DLF Seeds A/S (CVR: 62556013).
https://datacvr.virk.dk/enhed/virksomhed/62556013?fritekst=62556013&sideIndex=0&size=10
Thise Mejeri A.M.B.A. (CVR: 12425694).
https://datacvr.virk.dk/enhed/virksomhed/12425694?fritekst=12425694&sideIndex=0&size=10
AKV Langholt amba (CVR: 34914311).
https://datacvr.virk.dk/enhed/virksomhed/34914311?fritekst=34914311&sideIndex=0&size=10
KMC Kartoffelmelcentralen amba (CVR: 15230614).
https://datacvr.virk.dk/enhed/virksomhed/15230614?fritekst=15230614&sideIndex=0&size=10
Kopenhagen Fur a.m.b.a. (CVR: 15275413).
https://datacvr.virk.dk/enhed/virksomhed/15275413?fritekst=15275413&sideIndex=0&size=10
Vestjyllands Andel amba (CVR: 61729615).
https://datacvr.virk.dk/enhed/virksomhed/61729615?fritekst=61729615&sideIndex=0&size=10
Bornholms Andelsmejeri a.m.b.a. (CVR: 30220110).
https://datacvr.virk.dk/enhed/virksomhed/30220110?fritekst=30220110&sideIndex=0&size=10
Naturmælk amba (CVR: 17995030).
https://datacvr.virk.dk/enhed/virksomhed/17995030?fritekst=17995030&sideIndex=0&size=10
GASA NORD GRØNT A.m.b.A (CVR: 54550715).
https://datacvr.virk.dk/enhed/virksomhed/54550715?fritekst=54550715&sideIndex=0&size=10
Salling Grovvarer amba (CVR: 68736315).
https://datacvr.virk.dk/enhed/virksomhed/68736315?fritekst=68736315&sideIndex=0&size=10
Vejrup Andels grovvareforening (CVR: 45231712).
https://datacvr.virk.dk/enhed/virksomhed/45231712?fritekst=45231712&sideIndex=0&size=10
Nordsjællands Andels grovvareforening amba (CVR: 42737615).
https://datacvr.virk.dk/enhed/virksomhed/42737615?fritekst=42737615&sideIndex=0&size=10
Gasa Odense Frugt-Grønt amba (CVR: 10639476).
https://datacvr.virk.dk/enhed/virksomhed/10639476?fritekst=10639476&sideIndex=0&size=10
Danæg amba (CVR: 15311819).
https://datacvr.virk.dk/enhed/virksomhed/15311819?fritekst=15311819&sideIndex=0&size=10
Andels-Kartoffelmelsfabrikken Danmark A.m.b.a. + Andels-Kartoffelmelsfabrikken Sønderjylland (AKS). (CVR: 62818328).
https://datacvr.virk.dk/enhed/virksomhed/62818328?fritekst=62818328&sideIndex=0&size=10
Karup Kartoffelmelsfabrik A.M.B.A (CVR: 16217719).
https://datacvr.virk.dk/enhed/virksomhed/16217719?fritekst=16217719&sideIndex=0&size=10
Andelskartoffelmelsfabrikken Midtjylland A.M.B.A. (CVR: 38569317).
https://datacvr.virk.dk/enhed/virksomhed/38569317?fritekst=38569317&sideIndex=0&size=10
Bording Minkfodercentral A.M.B.A. (CVR: 31677513).
https://datacvr.virk.dk/enhed/virksomhed/31677513?fritekst=31677513&sideIndex=0&size=10
Holstebro Minkfodercentral A.m.b.A. (CVR: 36582014).
https://datacvr.virk.dk/enhed/virksomhed/36582014?fritekst=36582014&sideIndex=0&size=10
Sjællands Pelsdyrfoder (CVR: 19827186).
https://datacvr.virk.dk/enhed/virksomhed/19827186?fritekst=19827186&sideIndex=0&size=10
Minkfodercentralen Vilsund A.M.B.A. (CVR: 55929017).
https://datacvr.virk.dk/enhed/virksomhed/55929017?fritekst=55929017&sideIndex=0&size=10

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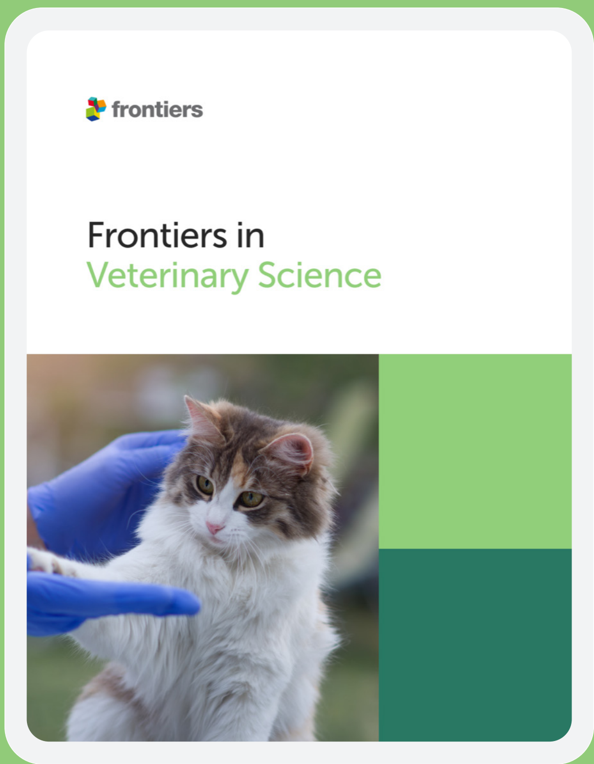

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