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The welfare of working animals

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The welfare of working animals

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Editorial: The welfare of working animals

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Editorial on the Research Topic
The welfare of working animals

This Research Topic gathers different contributions highlighting the latest research on the welfare of working animals. Articles ranged from working dogs, camelids, equids to heavy cattle, so we can say that contributors have fulfilled our intention, which was to identify problems, spread knowledge and seek solutions to further improve the welfare of all working animals. However, two groups of animals were more significantly represented, the first one being working dogs, and the other working equids.

Working dogs are prevalent around the world and fulfill many roles, adding social, cultural and economic value to human lifestyles. Dogs work in herding, guarding, hunting, human assistance, and animal-assisted therapy. In the last decade, our understanding of working dog performance and animal welfare science has grown rapidly. Despite this, there remains a great deal of room for further research, development, and improvement in working dog welfare (1).

In the first article in the working dog's group by Farr et al. a revised version of Penn Vet Working Dog Center Sprint Test is presented. This study illustrates the validity of assessing performance by naive and experienced raters, demonstrates that dogs accelerate similarly to humans, and discusses the performance of some working dogs. These outcomes are expected to establish the aforementioned sprint test as a valid measure of canine fitness, facilitate its use in future research, and enhance the medical care and welfare of canine athletes.

Earnshaw et al. have presented the first ever research examining the health of working dogs used in conservation in Africa. Authors emphasize that the importance of the health and welfare of these highly valuable dogs has been overlooked in the published literature as they require a unique skill set to be successful in their work. A strong handler-dog attachment, proficient handler training, and the acknowledgment of the challenging environment were pivotal to maintaining dog health. Finally, we believe that this very specific study will give additional help to those working in conservation programmes giving them a more comprehensive insight into what is required to establish and maintain a conservation dog programme.

The final two articles on the working dog welfare are focused on issues regarding their coat. On the one side, Discepolo et al. presents the problems of overbathing the working dogs and the potential health and welfare problems which can arise from this practice. The results of their presented work show that repetitive bathing of canines with detergent resulted in significant impacts on the resident dermal microbiota on canine skin. The study

by Perry et al. gives an additional insight into the issues regarding the health concerns associated with potential bacterial cross-contamination from working canines to humans. The authors have selected specific method of decontamination—wipe-down procedure, as it may be more readily utilized for canines working in healthcare setting to prevent the spread of bacteria, but the method can also be used easily in other scenarios, such as natural disasters. The study clearly states that both tested solutions (povidone-iodine and chlorhexidine gluconate) have similarly effective biocidal activity on the canine coat, both are easily available and approved for veterinary use, and easy to acquire and safe for utilization in canines.

Equids are still one of the most important resources for countless families around the world, who use them in a number of jobs, from cultivating fields to transporting goods (2). In this Research Topic, the welfare of working equids is represented by three articles, two research studies and a one systematic review. The cross-sectional study of donkey owners in Pakistan by Bukhari et al. provides evidence of on-the-ground working practices and factors associated with mounted load carrying, which is critical for developing evidence-based recommendations for loading, to improve the welfare of working donkeys. This study confirmed the necessity for future education of owners regarding the overloading as most donkeys reported in the study carried more than the recommended 50% limit of their bodyweight ratio.

The article by Cousquer et al. on the history and welfare of mules engages with systems thinking and presents their welfare as a complex interaction of several elements. Some of these elements are material, related to the animal itself and the tack, others are historical, geographical, socio-cultural, socio-economic and psychological. This article therefore deliberately sets out to highlight the emergent complexity of such relational systems and the need for our thinking to move away from linear cause-and-effect thinking and to embrace, instead, the systems approach with regard to One Health.

The review by Bukhari and Parkes focuses on the biomechanical, physiological, biochemical, and behavioral impacts of pulling load on equids and their welfare. Authors presents details all of the above-mentioned factors with special emphasis on the usage of simple indicators such as eye blink rate as one of the indicators of stress which could be used in the future assessments of overloading.

Kandeel et al. presented in their systematic review a bibliometric analysis of camel research. In their comprehensive article, the major contributors to camel research throughout the past century are discussed, along with the funding sources, academic institutions, scientific disciplines, and countries that contributed to the selected topic.

Finally, the last article by Gascho et al. focuses on the stunning of heavy cattle due to the problems that can occur at this sensitive moment due to their very thick frontal bones. In their research authors tested a new method and bullets for stunning heavy cattle. Based on the results of this study, the authors recommend two types of bullets for stunning heavy cattle with BigBovid - Hornady FTX and Hydra-Shok. These types of bullets have a high energy density and therefore a high penetration potential through the thick frontal bones of heavy cattle, secondly, excessive penetration is unlikely due to high fragmentation and thirdly at the relevant penetration depth, these two types of bullets caused adequate cavity volume and are therefore considered suitable for stunning heavy cattle.

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KN: Writing—review & editing. TM: Conceptualization, Writing—original draft, Writing—review & editing.

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ORIGINAL RESEARCH
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Welfare Concerns for Mounted Load Carrying by Working Donkeys in Pakistan

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Working donkeys (*Equus asinus*) are vital to people's livelihoods. They are essential for carrying goods, however, globally, overloading is one of the primary welfare concerns for working donkeys. We studied mounted load carrying by donkeys and associated factors in Pakistan. A cross-sectional study of donkey owners ($n = 332$) was conducted, and interviews were undertaken based on a questionnaire. Owners estimated that the median weight of their donkeys was 110 kg [interquartile range (IQR) 100–120 kg], and that they carried a median mounted load of 81.5 kg (IQR 63–99 kg). We found that 87.4% of donkeys carried a load above 50% of their bodyweight ratio (BWR), the median BWR carried was 77.1% (IQR 54.5–90.7%), and 25.3% of donkeys carried above 90% BWR. Donkeys that were loaded at more than 50% BWR were more likely to adopt sternal recumbency compared to donkeys loaded with less weight ($P = 0.01$). Donkeys carrying construction material were more likely to carry more than the median BWR, when compared to domestic loads ($P < 0.001$). Younger donkeys aged between one and 5 years carried more than the median BWR compared to those aged over 15 years ($P = 0.03$). For the models with donkeys carrying median BWR and above 90% BWR, those working in peri-urban and urban areas were more likely to carry a greater BWR than donkeys working in rural areas ($P < 0.001$; $P < 0.001$, respectively). For donkeys carrying more than 90% BWR, mixed breed donkeys carried higher loads compared to other breeds of donkeys ($P < 0.001$). Overloading based on current recommendations (50% BWR) was common, with the majority (87.4%) of donkeys reported to carry more than the recommended 50% limit. This survey provides evidence of on-the-ground working practices and factors associated with mounted load carrying, which is critical for developing evidence-based recommendations for loading, in order to improve the welfare of working donkeys.

Keywords: animal welfare, donkey welfare, loading practices, overloading of donkeys, working equids

INTRODUCTION

Donkeys have played an essential role in developing human civilizations (1). There are approximately 50.5 million donkeys globally (2), benefiting around 600 million people and playing a vital role in the livelihood of poor and vulnerable communities in lower-middle income countries (LMICs) (1, 3–7). The importance of working donkeys for their owner's livelihoods and the economies of developing countries is well known (3, 5, 7); for example, in Senegal, draft donkeys contribute 74% of their driver's annual income (8). However, research has not yet quantified the value of working donkeys to the overall economies of LMICs (7). This may be why their importance has often been overlooked in government-level animal welfare policies (9). As such little is done to safeguard donkey welfare (10), leading to compromised welfare due to harsh working conditions, lack of legislation, and marginalization of both donkeys and donkey owning communities (3, 7).

Donkeys are used in a variety of settings, across rural, peri-urban, and urban areas (10) for plowing, fallowing, cultivation, and human transportation (3, 11). Donkeys are also used as pack animals for the transportation of construction, agricultural products, and domestic loads (3, 5, 7), including brick production (**Figure 1**). One of the most severe problems working donkeys experience is overwork and overloading (12–14). Overloading can be defined as the amount of weight that disrupts gait rhythm, resulting in lameness and behavioral changes (7). Some of the most common welfare issues documented in load carrying donkeys are skin sores and lesions, poor physical condition, chronic back pain, exhaustion, wounds, fractures, heat stress, dehydration, sprains, lameness, colic, metabolic disorders, myopathies, fear of humans, infrequent feeding, and hypoglycemia (11, 15, 16). Donkeys carry tons of mounted weight every day, which likely exceeds their natural weight carrying ability (7), and they work for extended periods of time (3, 7, 17). It has been reported that donkeys work for up to 12 h a day in Ethiopia, and cover a distance of more than 30 km a day in Morocco (17, 18). The working schedule of donkeys in Pakistan is currently not documented.

Animal welfare can be defined as the state of the animal's body and mind, and the extent to which its nature is satisfied (19). High workload and unsafe practices can contribute to poor working donkey welfare (3, 7), as overloading has been identified as impacting on equid behavioral, biochemical, biomechanical, and physiological characteristics (7). Behaviors associated with heavy loads include a reluctance or refusal to move forward and falling down, constant movement of the head with ears back, aggression, defecation, ear lifting, tail swishing, sniffing and moving backward, heavy and rapid panting, and reduced responsiveness (7, 10, 15). Excessive mounted load causes a number of internal biochemical changes, for example, a rise in blood lactate, nitrates, nitrites, and cortisol concentration (7, 15). Overloading can also affect gait biomechanics in horses, as it disturbs stride parameters, gait stability and symmetry. It is not known yet if these effects are evident in donkeys but it is highly likely (7). Finally, loading induces changes in multiple physiological indicators, for example, increase in heart rate, respiration rate, rectal temperature, and hematocrit. Mounted load-associated work also induces changes in muscle composition. However, heart rate variability decreases with heavier mounted load compared to lighter weights (7, 16).

There is little research regarding mounted load-carrying limitations of working donkeys. The maximum load recommended for a fit donkey in the UK, with a well-balanced load, is 50 kg (20), which is approximately 28% of an adult donkey's bodyweight. This 50 kg recommendation is not evidence-based, and refers to donkeys in the UK, which typically are in good body condition and are larger (21) than working donkeys in LMICs (22). Current guidelines for working donkeys based on research carried out in India suggest that donkeys can safely carry loads of up to 50% bodyweight (23). Donkeys in some LMICs have been reported to carry as much as 75% of their bodyweight (24). However, there is evidence of donkeys carrying up to 117% of their body weight in Pakistan (7, 25). Even conservative estimates indicate that these Pakistani donkeys carry more than their own bodyweight, which is one of the causes of compromised welfare (7). An increase in weight of the mounted load causes an increase in prevalence of skin wounds in working donkeys (17). Previous research has identified more hoof and gait abnormalities, tendon issues, joint swelling and soft tissue injuries, in older compared to younger donkeys. Older donkeys also had lower body condition scores (26). The current study aimed quantify demographics of donkeys, donkey loading practices, and factors related to mounted load carrying, in order to identify factors that may impact donkey welfare in Pakistan.

MATERIALS AND METHODS

Study Area and Study Design

We carried out a cross-sectional survey of donkey owners in four of Pakistan's regions (Swat, Attock, Faisalabad, and Bahawalpur; **Figure 2**). These regions were selected based on their topography and varying climatic conditions: mountainous, arid, irrigated plains, and sandy desert, respectively (27). Different topographic regions were selected because working equids face different challenges in different communities and geographic sites (9, 28). The four regions cover almost 39,815 km^2 (almost 4.5% of the country) of Pakistan. Swat (34°45' latitude, 72°54' longitude) is a mountainous region with an elevation of 2,591 m above sea level. The maximum average monthly temperature (37°C) remains during July, and the minimum average monthly temperature (0°C) is recorded during January. In Swat, annual rainfall ranges between 1,200–1,400 mm (27). Attock (32°55' latitude, 72°51' longitude) is an arid and semi-hilly region with an elevation of 519 m above sea level. The maximum average monthly temperature (38°C) occurs in June, with the minimum average monthly temperature (3°C) recorded in January. In Attock, annual rainfalls range between 900–1,000 mm (27). Faisalabad (31°26' latitude, 73°08' longitude) is among the irrigated plains of Pakistan, with an elevation of 185 m above sea level. The maximum average monthly temperature (41°C) occurs in June, with the minimum average monthly temperature (5°C) in January. In Faisalabad, annual rainfalls range between

FIGURE 1 | Mounted load carrying by donkeys in a brick kiln production system in Pakistan. Photo: Syed S. U. H. Bukhari.

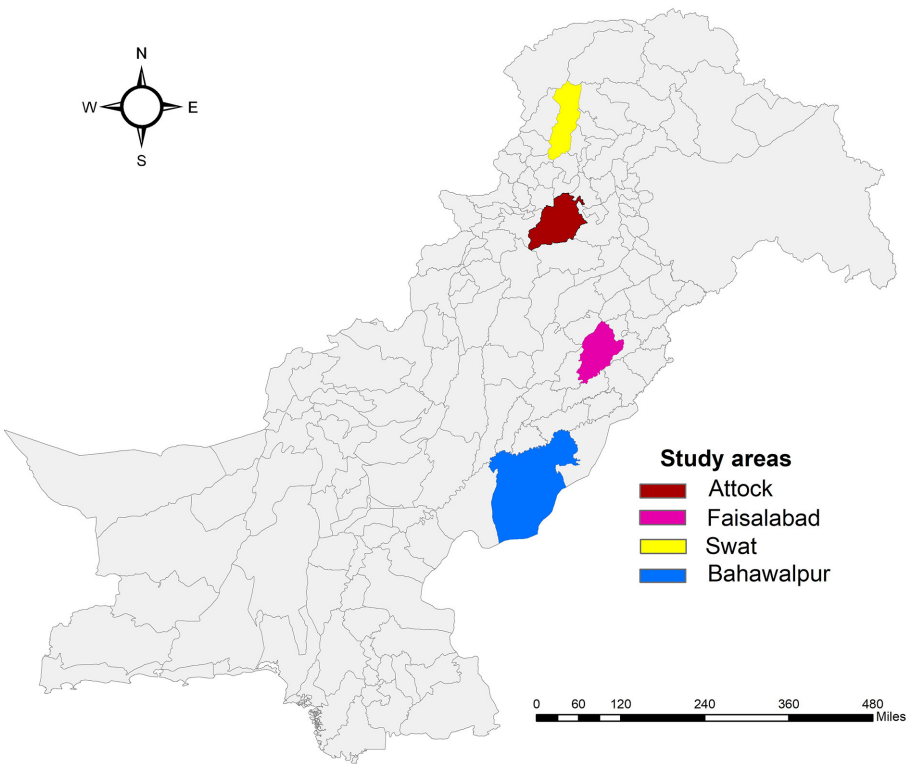

FIGURE 2 | A map of Pakistan showing the locations of the four regions in which the study was conducted.

300–400 mm (27). Most of Bahawalpur (28°39' latitude, 70°41' longitude) is a sandy desert region with an elevation of 88m above sea level. The maximum average monthly temperature (42°C) occurs in June, with the minimum average monthly temperature (4°C) in January. In this region, annual rainfalls range between 100–150 mm (27).

Questionnaire Design

A questionnaire was developed to assess demographic characteristics, practices, and factors associated with mounted load carrying. The questions were designed based on field conversations with donkey owners combined with recent field experience of registered equine veterinarians in the four selected study regions. The questionnaire consisted of both open-ended and closed questions. The first section of the questionnaire consisted of informed verbal consent of donkey owners. The second section included information regarding the demographics of the owner, and the signalment of the donkey. The third section contained questions on the loading practices. In this section we asked about the weight of the donkey, weight of mounted load, whether the donkey adopted sternal recumbency when loaded, type of saddle, type of load,

distance traveled, working terrain, working speed, daily working hours, signs of lameness, and daily income. The bodyweight of donkeys and the weight of any mounted loads as a part of their regular loading practices were estimated by the donkey owners. In some cases, donkey owners are able to weigh their donkeys on scales at nearby dairy farms or had recently weighed their donkey. Other owners estimated their donkey's weight. The weight of the mounted load was estimated depending on the items carried. For bricks, mounted load weight estimation was done by multiplying the number of bricks by the known weight of one brick. The weight of commercially packaged items was written on packaging, for example, a bag of cement, a bag of wheat grain, bags of fertilizers etc. The weight of liquids such as milk, oil, and water containers were estimated by number of liters in one can and the number of cans carried. Donkey owners were asked about sternal recumbency—whether the owner had previously noted a donkey trying to adopt sternal recumbency after loading—and lameness. The sternal recumbency and lameness could be observed by the owner without knowing the cause and to ensure clarity, the definition of sternal recumbency and lameness were explained if needed. Sternal recumbency was chosen as anecdotally, donkeys have been reported to perform this behavior under load.

A pilot study was conducted to optimize questions being asked, address discrepancies and check how much time the questionnaire took to complete. Information was collected from 24 randomly selected donkey owners, six from each of the four target regions (29). The time to complete the survey was 8–10 min. Surveys were all conducted verbally due to low literacy rates in the surveyed population. None of the data gathered from the pilot survey were included in the final analysis.

Data Collection

The survey was conducted by equine veterinarians. They verbally explained the study, its purpose, and its methods. The donkey owners were approached based on convenience sampling and willingness to participate. Donkey owners were identified for inclusion in the study at the work location and on the basis of interviewer knowledge of the owners at a local level. Then the age of the owner was determined verbally and if they were more than 18 years old, they were recruited for the interview. Their informed verbal consent was taken before the start of the interview. Once donkey owners had provided consent to participate, interviews were undertaken based on the pre-designed questionnaire. The number of donkeys per owner varied. If an owner had more than one donkey, he answered the questions for one donkey. A total of 332 donkey owners participated. They had the opportunity to ask questions, and all their questions were answered appropriately. The interviewer signed a "participant informed verbal consent form". A third person signed the witness statement (witness, to ensure appropriate exchange of information) on "participant informed verbal consent form" according to existing survey guidelines (30, 31). Face-to-face interviews were conducted to collect the required data, based on the pre-structured questionnaire which was in English. However, interviews were delivered in the local languages (Urdu, Pashtu, Hindko, Pothwari, Punjabi, Saraiki) after translation by the interviewers who were equine veterinarians and fluent in both English and the respective local languages. This approach was used to maximize the accuracy of responses and minimize any confusion concerning the scientific terminology used according to existing survey guidelines (30, 31). The questionnaire can be found in **Supplementary Table 1**.

Statistical Analysis

The continuous data (weight of donkey, weight of the load, daily income generated by the donkey, and distance traveled per day) were presented in the form of median, interquartile range (IQR), minimum, and maximum. All the categorical data were described as frequency and percentage.

Outcome Variables

The following formula was used to calculate the percent bodyweight ratios (%BWR) for all donkeys,

$$\%BWR = \frac{Weight\ of\ Mounted\ Load}{Weight\ of\ Donkey} \times 100 \quad (1)$$

Three new binary outcome variables were created and labeled (1) 50% BWR, (2) median %BWR and (3) high %BWR. The first binary outcome variable had a cut-off BWR of 50% and was selected as an outcome variable based on existing guidelines which suggest that a donkey can safely carry up to 50% of their bodyweight (23). The second binary outcome variable had a cut-off BWR was the median of percent BWR in the population investigated, with half of the donkeys carrying above the median %BWR. The third binary outcome variable had a cut-off BWR of 90% and represented the upper quartile of our study population.

Exposure Variables

The variables considered in the logistic regression models were area (urban, peri-urban and rural), donkey age, donkey sex, breed (Sperki, Shinghari, Indian and mixed breed), type of saddle used, working terrain (mixed, plains, steep) and speed (walking or trotting). Continuous variables were non-normally distributed and were included in the model as categorical variables based on quartiles. Continuous variables were distance covered per day (in km) and earnings of a donkey in Pakistan rupees (PKR). Two donkey behaviors, sternal recumbency when loaded (yes/no) and lameness signs while working (yes/no) were included. Donkey breed and age were further categorized as binary variables, i.e., mixed breed and other breeds during multivariable modeling.

Univariable and Multivariable Regression Models

Univariable and multivariable logistic regression models were used to determine explanatory variables associated with mounted loads. Three multivariable logistic regression models were developed to investigate factors associated with each of the outcome variables—high %BWR, median %BWR, and 50% BWR. Exposure variables were screened using univariable logistic regression model for each outcome variable. Univariable

regression models are provided in **Supplementary Table 2**. Exposure variables with a likelihood ratio test (LRT) *P*-value <0.20 were selected for inclusion in the multivariable model for that outcome. A preliminary multivariable model was built using a manual backward stepwise method of elimination in which variables were retained in the final multivariable regression model if the LRT *P*-value was <0.05. The LRT was used as the primary selection criterion. Confounding was assessed throughout the multivariable model building, with variables changing the odds ratio (OR) more than 10% retained in the final model. The goodness-of-fit of the logistic regression models was assessed using the Hosmer-Lemeshow test. All statistical analyses were conducted using Stata IC version 17 (32).

Ethical Approval

This study was approved by the Human Subjects Ethics Sub-Committee, City University of Hong Kong (Approval reference no. JCC2021AY003).

RESULTS

Demographic Characteristics

In total, 332 donkey owners agreed to participate. The demographics of the owners and donkey signalment are presented in **Table 1**. The majority of questionnaire participants (98.5%; $n = 327$) were men. Both male (54.5%; $n = 181$) and female (45.2%; $n = 150$) donkeys were used for load-carrying work. The majority of donkeys (58.1%; $n = 193$) were aged between 6 to 10 years. Donkeys worked in rural (48.7%; $n = 162$), peri-urban (38.3%; $n = 127$), and urban (13.0%; $n = 43$) areas. The distance covered by donkeys during their working day was a median of 8 km (IQR 3–17 km). Daily earnings were a median of 685 PKR (IQR 450–900) (USD$3.87 (IQR $2.54–$5.08)).

TABLE 1 | Demographic characteristics of the donkey owners and donkeys.

Variable	Category	Number	Percentage (%)
Owner age (years)	<31	75	22.6
	31–40	141	42.5
	41–50	97	29.2
	>50	19	5.7
Owner gender	Male	327	98.5
	Female	5	1.5
Area	Rural	162	48.8
	Peri-urban	127	38.3
	Urban	43	13.0
Age of donkey (years)	<6	38	11.4
	6–10	193	58.1
	11–15	62	18.7
	16–20	23	6.9
	>20	16	4.8
Donkey sex	Male	181	54.5
	Female	150	45.2
	Gelded	1	0.3
Donkey breed	Sperki	26	7.8
	Shinghari	49	14.8
	Indian	9	2.7
	Mixed Breed	248	74.7

Mounted Loads and %BWR

The median weight for donkeys was 110 kg (IQR 100–120 kg) and the median mounted load for one trip was 81.5 kg (IQR 63–99 kg) (**Figure 3**). The median %BWR was 77.10% (IQR 54.50–90.70%). Overall, 87.4% donkeys carried loads above 50% BWR. Twenty-five percent of donkeys carried loads above 90 %BWR (high %BWR).

Donkey Owners and Load Carrying

Owners reported 44.0% ($n = 146$) of donkeys were used for carrying construction-related material, 38.3% ($n = 127$) were used for carrying agricultural-related material and 17.7% ($n = 59$) were used for domestic goods. We found that 37.7% ($n = 125$) of donkeys were working on flat terrain, 5.4% ($n = 18$) on steep terrain, and 56.9% ($n = 189$) on combined flat and steep terrain. Most donkeys ($n = 321$, 96.7%) only walked during their routine daily work. In total, 41.6% ($n = 138$) of the donkey owners reported that they routinely saw lameness in their donkeys while working (**Table 2**).

Logistic Regression Modeling

Donkeys Carrying 50 %BWR

Distance traveled (km), breed of donkey, and sternal recumbency after loading were all retained in the final model. Mixed breed donkeys were 2.57 [95% Confidence Interval (CI) 1.21–5.46] times more likely to carry loads of more than fifty percent of their bodyweight, compared to other breeds of donkeys ($P = 0.01$). Donkeys traveling over 8 km per day were 7.16 (95% CI 1.47–34.79) times more likely to carry loads of more than 50% of their bodyweight, compared to donkeys traveling up to 3 km per day ($P = 0.01$). Donkeys were 4.20 (95% CI 1.30–13.55) times more likely to adopt sternal recumbency when loaded if they were loaded with more than 50% of their bodyweight, compared with donkeys loaded with less weight ($P = 0.01$) (**Table 3**).

Donkeys Carrying Median %BWR

Area, age of donkey, type of load, earnings per day (PKR), and distance traveled (km) were all retained in the final model. The odds of carrying a load of more than the median %BWR in the sampled population of donkeys was higher if the donkey was working in a peri-urban [OR 2.78; 95% CI (1.16–6.63)] or urban area [12.82 OR; 95% CI (3.68–44.71)], compared to a rural area ($P = <0.001$). Younger donkeys aged between 1 and 5 years carried more than median weight compared with donkeys aged 15 or older [11.38 OR; 95% CI (1.10–117.20); $P = 0.03$]. Donkeys carrying construction materials were more likely to carry over the median BWR [OR 5.41; 95% CI (1.69–17.26); $P = 0.004$], (**Table 4**).

Donkeys Carrying High %BWR (90% BWR)

Area, age of donkey, breed, working terrain, and working hours per day were all retained in the final model. The odds of carrying a load of more than 90% of bodyweight was higher if the donkey

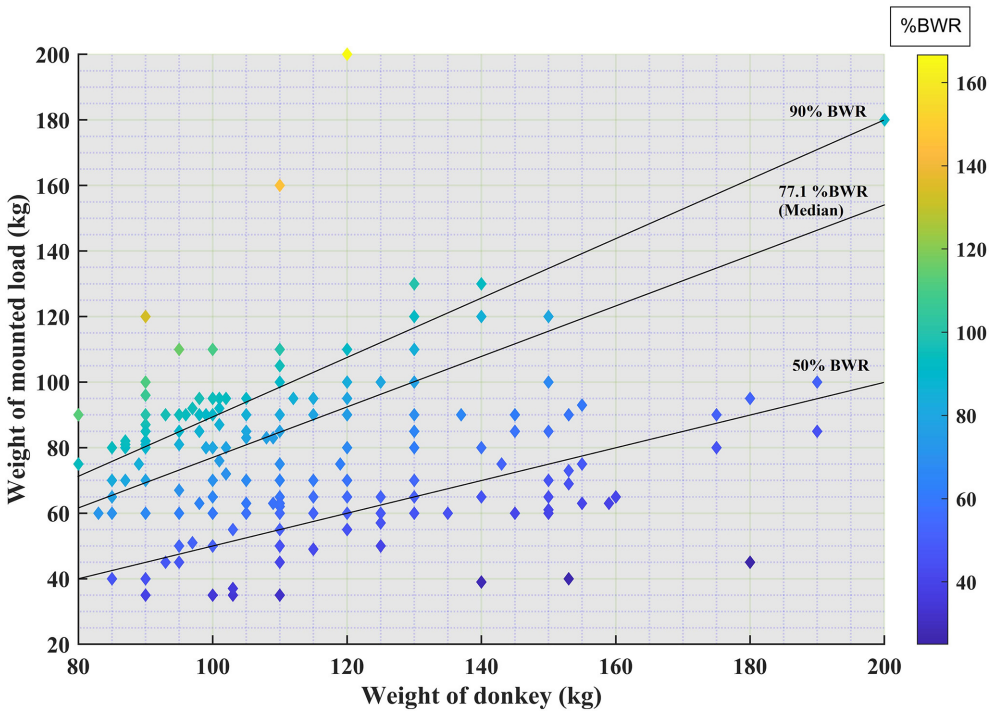

FIGURE 3 | Donkey weight plotted against load carried. Lines represent 50% bodyweight ratio (BWR) carried, the median %BWR and high BWR (the upper quartile for %BWR). Lighter colors represent a higher %BWR.

was working in a peri-urban [OR 14.51; 95% (CI) (4.10–51.37)] or urban area [OR 8.38; 95% CI (2.15–32.67)], compared to rural areas ($P \leq 0.001$). Mixed breed donkeys were 17.92 (95% CI 2.40–133.87) times more likely to carry loads of more than 90 percent of their bodyweight, compared to other breeds of donkeys ($P = 0.005$). Donkeys working for more than 8 h a day were 26.31 (95% CI 4.11–168.52) times more likely to carry a load more than the 90% BWR cut-off, compared to donkeys working <5 h per day (**Table 5**).

DISCUSSION

Studying load carrying in working donkeys is important because high workload and unsafe practices contribute to poor working donkey welfare as it leads to gait disruption, ataxia, the development of abnormal behaviors, lameness, and soft tissue and bone injuries (3, 7). We explored mounted load carrying by donkeys in Pakistan and the factors associated with the weight of load carried. Despite overloading being an important donkey welfare problem (7), the current report is the first to elucidate factors associated with mounted loads carried under field conditions. One quarter of donkeys carried loads equal to 90% or more of their own bodyweight, with some donkeys estimated by their owners to be carrying more than 150% of their bodyweight. Factors including the type of load carried, the breed and age of the donkeys, and their location of work were associated with how much load the donkey carried. The variables (type of load, age of donkey, daily working hours, distance traveled and area of work) associated with donkey loading in this population have previously been associated with the poor welfare of working donkeys more broadly (7, 17, 18, 26, 33–35).

Overloading based on current recommendations (50% BWR) (23) was common, with the majority (87.4%) of donkeys reported to carry more than 50% BWR. The weight of donkeys in our study was comparable to a previous report of draft donkeys in Pakistan (36). The weight of mounted loads found in our research is also similar to previous investigations from Ethiopia (17) and India (37). However, it is suggested that donkeys can carry more than one third of their bodyweight (i.e., 40–80 kg) (38). Further, experimental research has suggested that donkeys can travel further, for longer and with less physiological impact if they are loaded with 40–50% of their bodyweight as compared to 66% of their body weight (23). Moreover, guidelines for donkeys working on beaches in the United Kingdom mandate carrying not more than 28% of bodyweight (24), however, these guidelines are not based on experimental evidence. As compared to donkeys, maximum permissible load-carrying limits suggested (based on experimental research) for native Japanese horses is 29% (39, 40), for Yonagunai ponies is 33% (41), and for Taishuh ponies is 43% of their bodyweight (40). Anecdotally, it is said that a rider should not weigh more than 10% of the horse's bodyweight in the UK, but in the US, this limit is doubled to 20% of the horse's weight; however, these guidelines are traditional rather than research-based, often impractical, and are seldom adhered to (24).

TABLE 2 | Practices of working donkey owners related to mounted load carrying.

Variable	Category	Number	Percentage (%)
Does your donkey sometimes adopt sternal recumbency after loading?	Yes	144	43.4
	No	188	56.6
What is the type of saddle you use for loading your donkey?	Wooden	162	48.8
	Cloth	31	9.3
	Plastic	4	1.2
	Hessian	133	40.1
	Don't use saddle for loading	2	0.6
Type of Load?	Construction material	146	44.0
	Agricultural load	127	38.3
	Domestic use	59	17.8
What is the working terrain?	Flat	125	37.7
	Steep	18	5.4
	Both flat and steep	189	56.9
What is the working speed?	Walk	321	96.7
	Trot	11	3.3
What are the working hours per day?	< 5	156	47.0
	5–8	154	46.4
	>8	22	6.6
Have you noticed lameness signs while working?	Yes	138	41.6
	No	194	58.4

In urban areas, donkey owners were more likely to load their donkeys to more than the median and more than 90% of their bodyweight compared to rural areas. The area a working donkey lives in is a known factor for poor working equine welfare (10, 18, 26), as rural donkeys usually had fewer lesions on their body than urban donkeys and a larger proportion of urban donkeys showed moderate to severe gait deviation (i.e., lameness) than rural donkeys (10, 42). Moreover, rural donkeys work less than those in urban areas (5). Unfortunately, these authors did not define "work less" in terms of a lighter loaded weight, shorter working hours or distance traveled. However, this finding may be why fewer welfare concerns were raised for rural donkeys (10). Furthermore, donkeys in rural and urban settings have different roles within these communities and face different welfare challenges (5, 10). Due to the differences in practices between urban and rural areas, determining the welfare and socio-economic value of working donkeys in different parts of the same territory is crucial.

The type of load carried (construction, agricultural, or domestic), was associated with the weight of mounted load. Donkeys working for the transportation of construction-associated load carry more weight than donkeys carrying agricultural loads. There is currently no research comparing the type of load carried and the weight of that load. However, working donkeys that transport different types of loads experience different impacts on their health and welfare (10, 26, 33, 34, 43–46). For example, donkeys used in brick transport are 2.5 times more likely to have moderate to deep skin lesions and 3.4 times more likely to have sole surface abnormalities than those used for other purposes (26). We hypothesis that because brick is a dense material, more bricks will fit on the back of a donkey than other materials, leading to heavier loads being carried when compared to less dense agricultural or domestic loads.

Most donkeys worked for <8 h and covered a median distance of 8 km (ranges, 1–30 km) per day. The daily working hours of donkeys varies in Ethiopia (29), Mexico (33), Egypt (34), and Nepal (46). In our study, donkeys working for a greater number of hours and covering more distance per day carried more weight. This is associated with the type of workload; donkeys working for the transportation of construction associated load usually carry more weight, work for longer hours, and cover more distance than donkeys working for domestic or agricultural work, as they typically carry less, for a shorter period of time and over a shorter distance. Donkeys transporting agricultural load work less than donkeys involved with other types of work (5, 26). Moreover, donkeys work for up to 12 h a day in Ethiopia (17), and cover a distance of more than 30 km a day in Morocco (18). As the duration of work and distance traveled increases, it compromises donkey welfare (17, 18, 34). Longer working hours and increased distance covered in addition to high mounted loads are likely to lead to fatigue, and fatigue from overworking and overloading can compromise donkey welfare and productivity (34), as fatigue, heat stress, and dehydration disturb body processes and can result in organ damage and even death (47, 48). Dehydration prevents thermal conductance from the core to the periphery, increasing the risk of hyperthermia (49). Hyperlactatemia and hypercapnia induce cardiac arrhythmias during work, which can result in cardiovascular morbidity and mortality. Reduced vascular integrity caused by hypovolemia can result in peripheral edema, pulmonary edema, laminitis, and intravascular clotting (48).

Donkey age was associated with load carried in all models when donkeys were carrying more than 50% of their bodyweight. Younger animals between 1 and 5 years of age carried more load compared to older animals. In the UK it is recommended that donkeys be at least 4 years old before starting work (20). Donkeys may appear mature at the age of two, but they are not skeletally mature until they are 3–4 years old, and it has been suggested that donkeys should not carry weight or work until they are 5–6 years old to avoid osteoarthritic changes due to overworking (50, 51). Previous research has found gait abnormalities, hoof abnormalities, tendon, joint swelling, and other load-associated injuries are more prevalent in older working donkeys (17, 26, 29); we suggest that this is because they have been carrying higher levels of mounted loads throughout their young lives, and they face multiple complex issues in their older age.

In our survey, 42% of donkey owners reported seeing lameness while their donkey was working. However, a more

TABLE 3 | Multivariable regression model for 50% body weight ratio (%BWR) of load carrying donkeys.

Variable	Level	Donkey carrying below 50 %BWR	Donkey carrying above 50 %BWR	Odds ratio (OR)	95% CI Lower	95% CI upper	Wald *P*-value	Likelihood Ratio *P*-value
Distance covered per day (Km)	<4	24	89	1				<0.001
	4–8	16	38	0.50	0.23	1.08	0.08	
	>8	2	163	7.16	1.47	34.79	0.01	
Donkey breed	Other breeds	24	60	1				0.01
	Mixed breed	18	230	2.57	1.21	5.46	0.01	
Does your donkey sometimes adopt sternal recumbency after loading?	No	38	150	1				0.008
	Yes	4	140	4.20	1.30	13.55	0.01	

TABLE 4 | Multivariable regression model for the median percent body weight ratio (%BWR) of load carrying donkeys.

Variable	Level	Donkey carrying below the median	Donkey carrying above the median	Odds ratio (OR)	95% CI Lower	95% CI upper	Wald *P*-value	Likelihood ratio *P*-value
Area	Peri-urban	22	104	2.78	1.16	6.63	0.02	<0.001
	Rural	135	28	1				
	Urban	9	34	12.82	3.68	44.71	6.26	
Donkey age (years)	1–5	11	27	11.38	1.10	117.20	0.04	0.03
	6–10	67	126	5.25	0.61	45.12	0.13	
	11–15	47	12	1.82	0.18	18.08	0.61	
	>15	41	1	1				
Type of load?	Domestic	51	8	1				<0.001
	Agriculture	91	36	3.03	0.88	10.34	0.08	
	Construction	24	122	5.41	1.69	17.26	0.004	
Earnings per day (PKR)	<700	55	111	1				0.005
	700–900	54	39	0.42	0.17	1.06	0.06	
	>900	59	14	0.18	0.06	0.53	0.002	
Distance covered per day (Km)	<4	102	11	1				<0.001
	4–8	43	11	2.15	0.70	6.57	0.18	
	>8	21	144	14.81	4.99	43.95	1.18	

in-depth lameness examination by a veterinarian or other appropriately trained professional would be needed to confirm this. In comparison, visual signs of lameness were observed in 15% of working equids by experts in Mexico (33), while gait abnormalities in working equids reported by experts in a wide range of countries range from 17.1 to 99.2% (26). A recent study of working donkeys pulling carts in the Faisalabad region of Pakistan found that 96% of donkeys were lame when examined by a veterinarian, despite examination being conducted while the donkey was still in harness (6). Owners in our survey reported less lameness as compared to previous reports from Pakistan; this could be due to differences in areas within Pakistan or may be due to donkey owner abilities to identify lameness. There is an assumption that owners report less lameness as compared to veterinarians and this assumption is based on surveys of horses, which have repeatedly demonstrated that owners report a lower prevalence of lameness and gait asymmetry than experts (52, 53). However, donkey owners have suggested work overload as a potential cause for lameness in Ethiopia (54) and Pakistan (6), and mule owners also recognize this issue (29). Lameness is one of the main welfare issues reported in working equids globally (6, 7, 10, 26, 29) and this is an area for important future targeted owner education. Donkeys are commonly presented with severe lameness due to their stoic demeanor, which can contribute to disease identification being delayed (55, 56). Clinical signs associated with lameness in donkeys in Pakistan includes tendinitis, joint swellings, reduced range of motion, pain on palpation, poor conformation, hoof abnormalities and back pain (36). In addition to poor welfare resulting from pain, lame horses expend more energy compared to sound horses when moving at a consistent speed. In case of animals working several hours per day, lameness may increase the demand on energy reserves in animals that already have low body condition scores (57). Alternatively, in order to compensate

TABLE 5 | Multivariable regression model for the high percent body weight ratio (%BWR) of load carrying donkeys.

Variable	Level	Donkey carrying below high %BWR	Donkey carrying above high %BWR	Odds ratio (OR)	95% CI lower	95% CI upper	Wald *P*-value	Likelihood ratio *P*-value
Area	Peri-urban	62	64	14.51	4.10	51.37	<0.001	<0.001
	Rural	159	4	1				
	Urban	27	16	8.39	2.15	32.67	0.002	
Donkey age (years)	1 to 10	149	82	28.72	4.82	171.01	<0.001	<0.001
	> 10	99	2	1				
Donkey breed	Other breeds	79	5	1				<0.001
	Mixed breed	169	79	17.92	2.40	133.87	0.005	
Working terrain	Mixed	151	38	1				<0.001
	Plains	83	42	0.31	0.14	0.67	0.003	
	Steep	14	4	223.54	14.56	3,431.07	<0.001	
Working hours per day	<5	151	5	1				<0.001
	5 to 8	86	68	11.95	2.48	57.55	0.002	
	>8	11	11	26.32	4.11	168.52	0.001	

for lameness, donkeys may work more slowly, resulting in lower productivity and decreased earnings for impoverished owners (58).

Donkey owners reported that donkeys carrying more than 50% of their bodyweight were more likely to adopt sternal recumbency after loading irrespective to type of load and area of work. However, the adoption of sternal recumbency requires further investigation in experimental research to determine its validity as a load quantifying factor in working donkeys. This study's two major limitations are convenience sampling and owner-reported weights, both of which are unavoidable in this context. Because the data are based on answers gathered during owner interviews, the accuracy of the data must be carefully considered (18). Furthermore, the reliability of 'owner information' has not been validated and may be imperfect or biased due to owner reporting of perceived "correct" answers (18).

Factors associated with mounted load carrying in working donkeys in Pakistan have been identified in this study. At present, there are no evidence-based guidelines for load carrying limits in working donkeys (7), although guidelines for pulled load exist in some countries (8). This survey is a starting point for the development of evidence-based recommendations for donkey loading. Quantified loading thresholds or predictors of overloading can then be used by non-governmental organizations (NGOs), legislators, and other decision-makers working with working donkeys to restrict overloading and optimize donkey welfare.

It is clear based on the use and role of the donkeys in this study, it is likely that recommending that they are only loaded at 50% of bodyweight will not be feasible. We need a greater understanding regarding the motivations and perceptions of owners around donkey loading, and the socio-economic role that load carrying donkeys play for their owners. While the welfare of the donkey is important, and a consideration here, donkey welfare can only be improved alongside community recognition of the issue, and a general improvement of human living conditions.

CONCLUSION

Our research has provided valuable information on the demographics of working donkeys, and the factors associated with mounted load carried by working donkeys in Pakistan. Factors including type of load carried, the breed, sternal recumbency, and age of the donkeys, and their location of work were associated with how much load the donkey carried. Overloading based on current recommendations (50% BWR) was common, and 87.4% of donkeys were carrying more than 50% of their bodyweight in the survey region. As overloading is one of the most common welfare issues in working donkeys, this is an area in which future education efforts should be targeted.

DATA AVAILABILITY STATEMENT

Due to data privacy concerns and conditions of the verbal consent given by the owners, the full dataset is not publicaly available. Queries can be made to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Human Subjects Ethics Sub-Committee, City University of Hong Kong (Approval Reference No. JCC2021AY003). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

All authors were involved in the preparation of the manuscript, gave final approval of this manuscript, read, and agreed to the published version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2022.886020/full#supplementary-material

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Ballistic study on the penetration potential and injury potential of different bullet types in the use of a newly developed bullet shooting stunner for adequate stunning of heavy cattle

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Introduction: Recently, a special bullet shooting stunner for heavy cattle has been developed that fires a bullet instead of a bolt. In the search for a suitable ammunition, the following criteria must be met: First, the energy of the bullet must be sufficient to penetrate the thick frontal bones of heavy cattle. Second, the injury potential at the corresponding penetration depth should preferably be large in order to damage brain tissue relevant to stunning. Third, the bullet must not perforate the occipital bone (over-penetration).

Methods: Four different bullet types [*Hornady FTX*, *Hydra-Shok*, *Black Mamba*, and a common full metal jacket (*FMJ*) bullet] were evaluated in a series of experiments on soap blocks and removed bone plates followed by computed tomography examinations. Penetration potential was evaluated in terms of kinetic energy relative to the caliber of the bullet, i.e., mean energy density (*ED*). Injury potential was evaluated by the mean extent of the cavity volume (e_{CV}) at the relevant penetration depth of 5.5 to 7.5 cm in the soap block.

Results: All four bullet types passed through the frontal bone plate. The *ED* was 17.50 J/mm^2 (*Hornady FTX*), 17.46 J/mm^2 (*Hydra-Shok*), 13.47 J/mm^2 (*Black Mamba*), and 13.47 J/mm^2 (*FMJ*). The *Hornady FTX* and the *Hydra-Shok* each fragmented heavily. The *FMJ* was excluded after three experiments due to over-penetrations. The e_{CV} was $e_{CV} = 3.77$ cm^2 (*Hornady FTX*), 2.71 cm^2 (*Hydra-Shok*), and 1.31 cm^2 (*Black Mamba*), with a significant difference ($p = 0.006$) between the *Hornady FTX* and the *Black Mamba*.

Discussion: For use in heavy cattle, the *Hornady FTX* and the *Hydra-Shok* are recommended due to the larger e_{CV} than the *Black Mamba*.

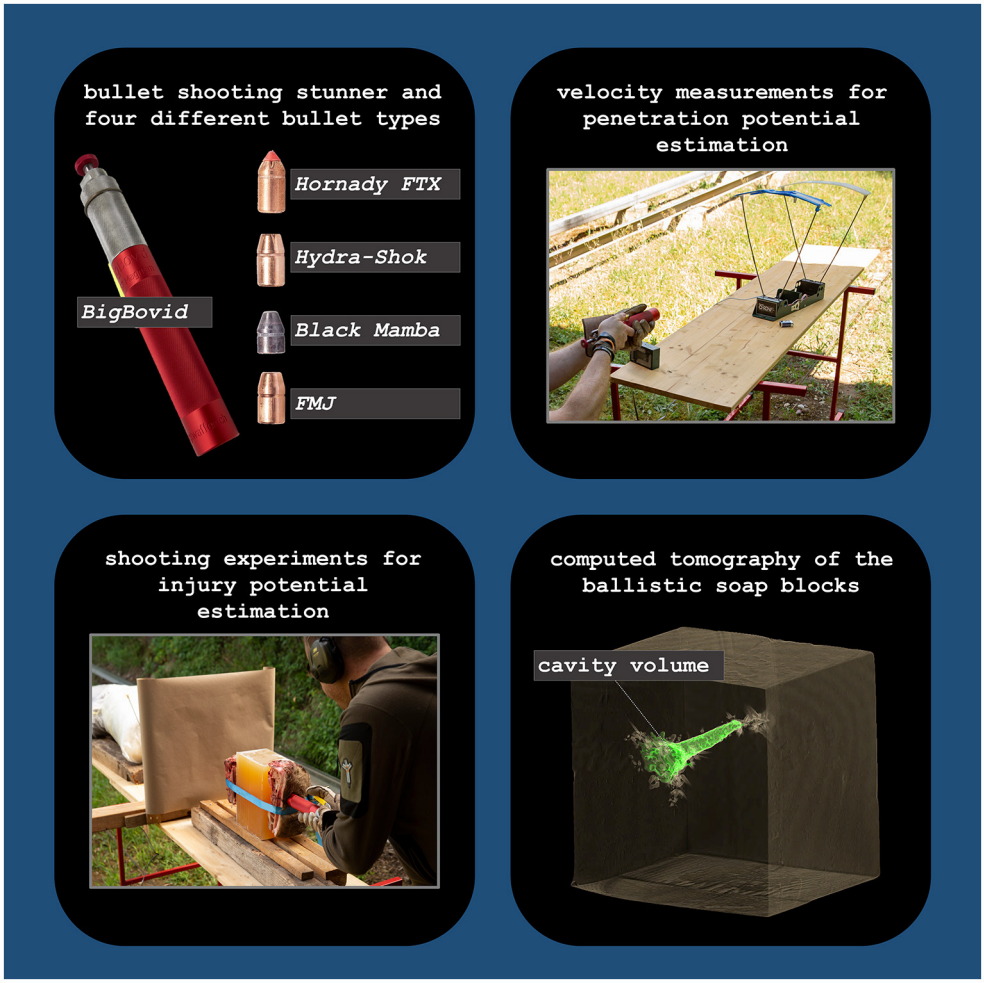
KEYWORDS

stunning effectiveness, concussion, animal welfare, slaughter, heavy cattle, *BigBovid*, *Humane Killer*, injury potential

Introduction

For stunning of cattle, a captive-bolt gun is typically used to damage brain regions that have critical functions for consciousness and the sensation of pain. The goal here is that the animal is no longer sensitive to pain, which is why the term "desensitization" is used for the intended effect of stunning (1). The brain structures to be destroyed are located in the region of the brain stem and thalamus (2), and thus centrally located deep in the brain. A bolt length of 120 mm is sufficient to damage the corresponding brain regions of cattle, except in water buffaloes due to their large frontal sinuses and occasionally in heavy cattle due to their larger skull size (3, 4). In addition, ordinary captive-bolt guns may be insufficient to punch through the frontal bones of heavy cattle with a live weight of up to 1,500 kg, so that adequate stunning is not assured. A previous study (5) has shown that beef bulls and older cull bulls are at higher risk for inferior stunning quality compared to dairy cows, female beef classes, or steer cattle classes. Improper stunning at slaughter causes pain and distress to animals, raising animal welfare concerns (6). To overcome problems in slaughtering water buffaloes and heavy cattle due to the insufficient bolt length and penetrating power of conventional stunning equipment, ordinary handguns are often used (4, 7). Although an improvement in the quality of water buffalo stunning has recently been demonstrated by the use of a pneumatic bolt gun (operating pressure: 200–220 psi; length of ejected bolt: 90 mm) at a newly proposed entry point located 8 cm above the reference point for cattle, a handgun ultimately had to be used in individual cases, even after a second follow-up shot with the bolt (8). In addition, such pneumatic bolt guns are not financially feasible for smaller slaughterhouses.

Since the use of ordinary handguns is not in the sense of our *Federal Food Safety and Veterinary Office* a newly developed 9 mm bullet-shooting stunner, named *BigBovid*, was recently presented for adequate stunning of heavy cattle (3). Instead of a bolt, this device fires 9 mm bullets to damage the corresponding brain regions. In this study (3), two different types of ammunition were used, a light full metal jacket (*FMJ*) truncated cone *.38 Special* (*Black Mamba*) and a semi-jacketed *.357 Magnum* with a soft tip (*Hornady FTX*). Based on three velocity measurements and the mass of the bullet, the mean kinetic energy of the respective bullet type when fired with the *BigBovid* was calculated (*Black Mamba*: 527.69 J, *Hornady FTX*: 1133.63 J). As a common measure for the



GRAPHICAL ABSTRACT

penetration potential in ballistics, the respective energy density (ED), i.e., the kinetic energy per unit reference area, was also given (*Black Mamba*: 8.18 J/mm^2, *Hornady FTX*: 17.56 J/mm^2), since this quantity is considered proportional to the penetration depth of a bullet into the tissue (9, 10). For handguns, the caliber squared of the bullet is usually considered the reference surface (9, 10). However, deformation and fragmentation can considerably change the reference area of the bullet, which then affects the penetration depth (9, 10). Since a bullet for adequate stunning of heavy cattle must pass through their thick frontal bones to reach the relevant brain regions, it can be assumed that, with the *Black Mamba* and *Hornady FTX* used, a deformed or fragmented bullet will penetrate the brain tissue. This was shown in the computed tomography (CT) examinations of the severed heads of the heavy cattle, which were stunned with the *BigBovid* (3). The data showed that the *Black Mamba* penetrated deeper into the tissue on average and over-penetrations were observed. The *Hornady FTX* fragmented in each case. Since both types of bullets perforated the frontal bones and reached the relevant brain regions, the recommendation was made for the *Hornady FTX* because of the distribution of fragments and because no over-penetration was observed. However, this alone does not allow a valid statement to be made about the effectiveness of the individual bullet types, since the respective injury potential could not be determined. For a solid recommendation of a bullet type and regulatory endorsement, the potential for injury to the brain regions relevant to stunning is critical. In addition, the bullet type evaluated and finally endorsed should be easily available.

The injury potential of interest depends on how much of the kinetic energy is eventually transferred to the brain tissue at the relevant section of the penetration depth (9, 10). This can be expressed indirectly by the volume of the temporary cavity that is temporarily formed along the bullet path (9, 10). With the extension of this cavity per distance, the deformation forces cause the injury to the tissue, which is stretched and torn in the process. In wound ballistics, this is described as local energy transfer, which is represented by the extent of this cavity (9, 10). Therefore, the extent of the cavity volume at the relevant penetration depth is an indirect measure of the injury potential (9, 10). Glycerin soap is typically used in ballistic experiments as a soft tissue simulant to investigate the extent of the temporary cavity. Such ballistic soap deforms mainly plastically, which means that the maximum extent of the temporary cavity in the soap block is "frozen" and consequently can be well studied and analyzed (10).

The objective of this study was to assess the injury potential of the different bullet types when shot with the *BigBovid* based on the cavities created in ballistic soap after passing through frontal bone plates from heavy cattle. In addition to the bullet types previously used (*Hornady FTX* and *Black Mamba*), two additional bullet types were evaluated in this study. First, the *Hydra-Shok* as an alternative to the *Hornady FTX*, due to its limited availability, and second, an ordinary full metal jacket bullet compared to the full-jacketed *Black Mamba*, in order to point out the increased danger when using an ordinary full metal jacket bullet. For this purpose, shooting experiments were performed and subsequent CT scans of the soap blocks allowed quantitative study of the cavities created by the individual bullet types at the corresponding penetration depth. Accordingly, this study presents the relevant ballistic characteristics of four different bullet types when used with the *BigBovid* and provides an assessment of the injury potential, respectively, the stunning potential when using the *BigBovid* in the slaughter of heavy cattle.

Materials and methods

Bullet specifications

Four different types of 9 mm bullets were tested for the use with the *BigBovid* bullet-shooting stunner (*Vogt Waffen AG, Oberglatt, Switzerland*). The four bullet types are shown in Figure 1. The first type of bullet was the *Hornady FTX*, a *.357 Magnum* (*Hornady® LEVERevolution®, Grand Island, Nebraska, U.S.A.*) with a mass of 140 grains (gr) and a special tip (*Flex Tip® Technology*) to transfer more energy than conventional bullets with a flat tip. The second type of bullet was the *Hydra-Shok* (*Federal Premium® Ammunition, Anoka, Minnesota, U.S.A.*), a readily available ammunition not previously used with the *BigBovid*. This hollow point bullet with a mass of 158 gr and a notched jacket is designed for controlled expansion when penetrating tissue. The third type of bullet was the *Black Mamba*, a full metal jacket truncated cone *.38 Special* (*Black Mamba*, *Fiocchi Ammunition, Lecco, Italia*), which has a slight curvature toward the inside at the flat tip and, in addition, this curvature toward the inside also has only a thin layer of the jacket. The *Black Mamba* is an effective hunting bullet with a low mass of 110 gr. The fourth bullet type was a conventional full metal jacket *.357 Magnum* (*Sellier* and *Bellot, Vlašim, Czech Republic*). This type of bullet, referred to as *FMJ* in this study, was selected to demonstrate potential danger of a conventional full metal jacket bullet when used for stunning cattle in an abattoir. It was assumed that the penetrating depth of this type of bullet with a mass of 158 gr is far too high and over-penetration is very likely. All bullets were fired by one and the same person using the *BigBovid*. The different types of bullets were evaluated in two separate experiment series.

Experiment series A

The first series of experiments (experiment series A) served to determine the kinetic energy and energy density, the basic data for bullet types used with the gun in question. These quantities were also determined for the bullet types already classified in the earlier study (3), since the data already collected were based on only three velocity measurements.

In the present study, velocities of the bullets were measured at a distance of approximately 50 cm from the barrel using a ballistic chronograph (*Model M-1, Shooting CHRONY Inc., Mississauga, Canada*). This distance was chosen to avoid erroneous measurements due to the expelled propellant gas. In the Supplementary material the experimental setup is illustrated. The measured velocity of the bullet was considered the muzzle velocity, i.e. the velocity the bullet has immediately after leaving the barrel. For each type of bullet ten velocity experiments were performed. From the velocity v and the mass m of the bullet, the kinetic energy

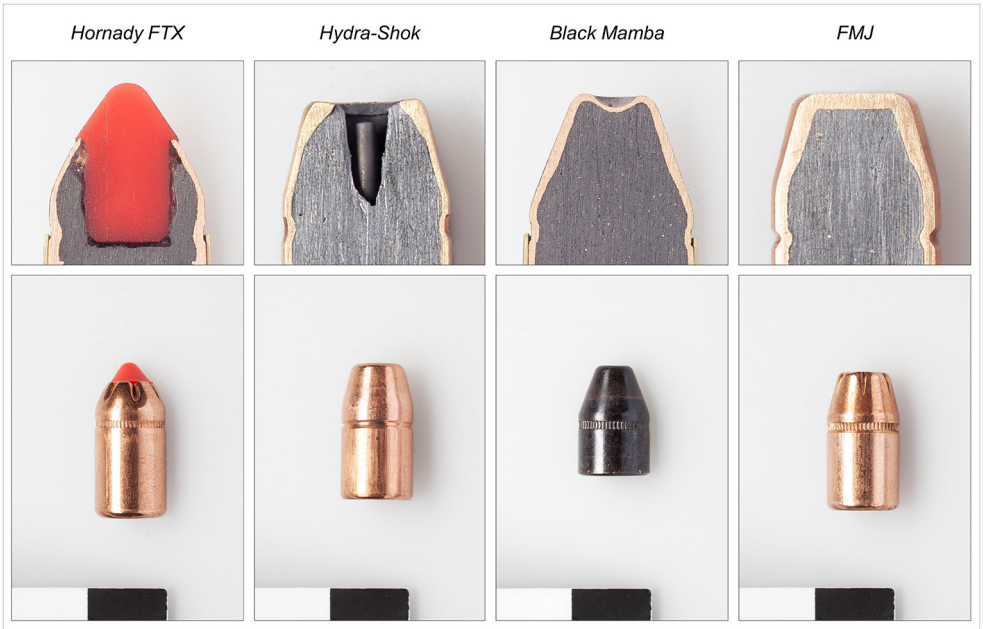


FIGURE 1
The four different 9 mm bullets evaluated in this study in terms of their injury potential when used with the *BigBovid* for stunning heavy cattle. Note the different design of the tip and the length of each bullet. The *Black Mamba* (110 gr) is shorter and has a lower mass than the *Hornady* (140 gr), the *Hydra-Shok* (158 gr), and the *FMJ* (158 gr).

E in joule (J) was calculated as follows:

$$E = \frac{1}{2}\, m\, v^2 \tag{1A}$$

For the mass of the corresponding bullet type, the value of the manufacturer's weight specification was used in the equation.

In ballistics, the kinetic energy density ED (also abbreviated as KED or E') in J/mm^2 is typically expressed as the kinetic energy per cross-sectional area A, where the cross-sectional area can be calculated from the caliber of the bullet (cal) (10):

$$d_{\max} \sim ED = \frac{E}{A} = \frac{2\, m\, v^2}{cal^2\, \pi}. \tag{2A}$$

For the caliber of all bullet types, a value of 9.07 cm (the conversion value of 0.357 inches rounded to two digits) was used for the calculations of the energy densities.

To account for the propagation of uncertainty in the calculation of the mean kinetic energy $\overline{E}$ and the mean energy density $\overline{ED}$, the mean error of the kinetic energy ($\Delta\overline{E}$), and the mean error of the energy density ($\Delta\overline{ED}$) was determined as follows:

$$\Delta\overline{E} = \frac{2\,\Delta\bar{v}}{\bar{v}}\bar{E}, \tag{1B}$$

respectively,

$$\Delta\overline{ED} = \frac{1}{A}\Delta\overline{E} \tag{2B}$$

where $\Delta\bar{v}$ is the mean error of the velocity measurements, which was calculated by the standard deviation of the velocities divided by the square root of the number of measurements.

Experiment series B

In the second series of experiments (experiment series B) the cavity volume created when the bullet penetrated the soap block was investigated. The extent of the cavity over the relevant section of the penetration depth was the measure for the injury potential.

For this purpose, contact shots were performed on ballistic soap blocks (*Mettler-Seifen SA, Henniez, Switzerland*) to which postmortem removed skull bone plates from heavy cattle were attached, namely the frontal bone plate at the front end of the soap block and the corresponding occipital bone plate at its rear end. The soap blocks had a dimension of approximately 25 × 25 × 20 cm and a weight of 13.5 kg. The bone plates were fixed to the soap block with a tension belt. A new pair of bone plates was used for each experiment. For the shot, the *BigBovid* was placed on the frontal bone plate. In the Supplementary material the experimental setup is illustrated. Eight contact shot experiments were intended for each type of bullet. Over-penetration, i.e., penetration of the occipital bone plate and onward flight of the bullet, was considered a major hazard in actual use of the *BigBovid*. Therefore, a bullet type that over-penetrated three times was excluded from further experiments.

After these shot experiments, the soap blocks were packaged and transported to a clinical CT scanner (*SOMATOM® Definition Flash, Siemens Healthineers, Erlangen, Germany*), where CT scans of the individual soap blocks were performed. The scan parameters were 120 kV, 400 mAs, and a pitch of 0.35 for reconstructions with an almost isotropic voxel size of approximately 0.6 mm^3 using a soft kernel (Br38) and a hard kernel (Br60). In addition, reconstructions with extended CT scale were made to visualize

TABLE 1 Results of experiment series A given as mean value (standard deviation) for *v* and mean value (mean error) for *E* and *ED*.

Type	*v* (m/s)	*E* (J)	*ED* (J/mm^2)
Hornady FTX	499.13 (7.74)	1130.28 (11.09)	17.50 (0.17)*
Hydra-Shok	469.32 (6.10)	1127.71 (9.28)	17.46 (0.14)
Black Mamba	377.39 (6.78)	507.74 (5.77)	7.86 (0.09)
FMJ	412.17 (5.53)	869.79 (7.25)	13.47 (0.11)

v, velocity; *E*, kinetic energy; *ED*, energy density. *w/o outlier: 17.64 (0.11).

TABLE 2 Results of experiment series B given as mean value (standard deviation).

Type	*d* (cm)	*V* (cm^3)	e_{CV} (δd) (cm^2)
Hornady FTX	18.50 (2.43)	54.34 (23.39)	3.77 (1.96)*
Hydra-Shok	17.38 (3.66)	42.13 (17.99)	2.71 (1.41)**
Black Mamba	18.42 (3.15)	18.31 (5.82)	1.31 (0.33)

d, penetration depth; *V*, total volume of the cavity; e_{CV} (δd), cross sectional extent of the cavity volume over the penetration depth section of 9–10.5 cm. w/o outlier: *3.22 (1.29), **2.30 (0.84).

the lodged bullets and bullet fragments. The reconstructed CT data were visualized and analyzed using medical image registration software (*syngo.via VB30A_HF07, Siemens Healthineers, Erlangen, Germany*). The total volume (V) of this cavity was measured with a region-based image segmentation method (region growing). The penetration depth (d) was measured using a linear distance measuring tool.

To estimate the injury potential, the mean extent of the cavity volume along the relevant section of the penetration depth was measured. The relevant penetration depth was derived from mean values of CT-based distance measurements ($\bar{s}$) between the skin and the thalamus ($\bar{s} = 10.2$ cm) and between the skin to the inner table of the frontal bone ($\bar{s} = 3.65$ cm) in cattle older than 30 months (4). Taking the difference of the mean distances, the relevant penetration depth from the inner table of the frontal bone to the thalamus was 6.55 cm. Since neither standard deviations of the measurements nor the exact number of CT measurements were given, a deviation of 1 cm was assumed. To estimate the corresponding mean extent of the cavity volume, cross-sectional images with 0.5 cm slice thickness were reconstructed from the CT data along the bullet path starting from the entrance hole. Then, the cross-sectional area (A) of the cavity volume was measured on CT images No. 11–15 corresponding to a penetration depth of 5.5–7.5 cm, respectively. A software-based freehand region-of-interest measurement tool was used for these volume measurements. Finally, the mean cross-sectional extent of the cavity volume (e_{CV}) in cm^2 was calculated along the relevant section of the penetration depth (δd), which is proportional to the injury potential (IP):

$$IP \sim e_{CV}(\delta d) = \frac{1}{n}\sum_{i=1}^{n} A_i \quad (3)$$

where n is the number of images.

Statistical analysis

For the measured data (v, V, d) and for $e_{CV}(\delta d)$ the mean values (standard deviations) are given. For the calculated physical quantities (E, ED), the mean values (mean errors) are given. The mean values, standard deviations, and mean errors given in this study were rounded to two decimal places.

The Shapiro-Wilk test was used to test for normal distribution. Analysis of variance (ANOVA) was used to test whether the mean values of the bullet types differ from each other in terms E, ED, V, and $e_{CV}(\delta d)$. When the ANOVA test showed a significant difference, pairwise t-tests with *Bonferroni* correction were used as *post-hoc* tests to check between which bullet types there was a significant difference. A p-value of <0.05 was considered statistically significant. The p-values given are rounded to three decimal places, respectively, very small p-values are given as <0.001. An outlier is defined as such if the value is smaller than the first quartile by 1.5 times the interquartile range or larger than the third quartile by 1.5 times the interquartile range. The statistical analyses were done in *RStudio (RStudio, Inc., Boston, MA, USA*).

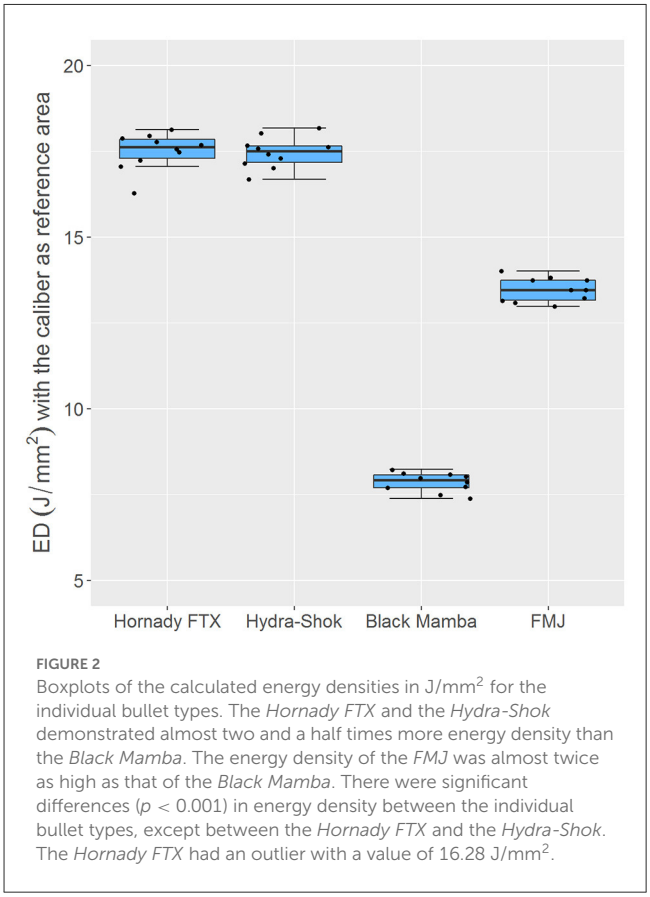


FIGURE 2
Boxplots of the calculated energy densities in J/mm^2 for the individual bullet types. The *Hornady FTX* and the *Hydra-Shok* demonstrated almost two and a half times more energy density than the *Black Mamba*. The energy density of the *FMJ* was almost twice as high as that of the *Black Mamba*. There were significant differences ($p < 0.001$) in energy density between the individual bullet types, except between the *Hornady FTX* and the *Hydra-Shok*. The *Hornady FTX* had an outlier with a value of 16.28 J/mm^2.

Results

Mean values are listed in Table 1 (experiment series A) and Table 2 (experiment series B). Individual values are listed in the

Supplementary material. In addition, there are CT reconstructions of all soap blocks in the Supplementary material.

Results of experiment series A

With a mean value of 7.86 (0.09) J/mm^2 the energy density of the *Black Mamba* was more than twice as low as that of the *Hornady FTX* at 17.50 (0.17) J/mm^2 and that of the *Hydra-Shok* at 17.46 (0.14) J/mm^2, and almost twice as low as that of the *FMJ* at 13.47 (0.11) J/mm^2 (Figure 2). The *Hornady FTX* had an outlier with a value of 16.28 J/mm^2. Excluding this outlier, the Hornady FTX had a mean energy density of 17.64 (0.11) J/mm^2.

The Shapiro-Wilk test showed that the kinetic energies and the energy densities of the *Hornady FTX*, the *Hydra-Shok*, the *Black Mamba*, and the *FMJ* were normally distributed. The ANOVA test revealed a significant difference between the bullet types in terms of their kinetic energy ($p = 0.002$) and in terms of their energy density ($p < 0.001$). The *post-hoc* tests revealed significant differences ($p < 0.001$) in the kinetic energy and the energy density between the *Black Mamba* and each other bullet type, and between the *FMJ* and each other type of bullet. There were no significant differences between the *Hornady FTX* and the *Hydra-Shok*.

Results of experiment series B

Soap block shootings were successfully carried out as each bullet perforated the frontal bone plate. However, while for the *Hornady FTX*, *Hydra-Shok*, and *Black Mamba* all eight experiments were performed without over-penetration, no further experiments were performed for the *FMJ* after three experiments, as this bullet also perforated the occipital bone sample on the back of the soap block. The over-penetrated *FMJ* formed a narrow channel in all three experiments with a total volume of 68.66, 44.89, and 42.76 cm^3, respectively. After passing through the frontal bone plate only tiny bullet fragments were visible along the narrow channel in the soap block (Figure 3). Due to the small number of experiments with the *FMJ*, this bullet type was excluded from the statistical evaluation.

The *Hornady FTX* and the *Hydra-Sho*k presented numerous fragments along funnel-shaped cavities that started from a large diameter immediately after the frontal bone sample and narrowed as the bullet penetrated the soap block. The *Black Mamba* exhibited few fragments along a narrow channel. The ANOVA test revealed a significant difference between the *Hornady FTX*, the *Hydra-Shok*, and the *Black Mamba* ($p < 0.001$). The mean volume of the *Black Mamba* was significantly smaller than those of the *Hornady FTX* ($p = 0.001$) and the *Hydra-Shok* ($p = 0.037$).

The mean extent of the cavity volume along the relevant section of the penetration depth was 3.77 (1.96) cm^2 for the *Hornady FTX*, 2.71 cm^2 (1.41) cm^2 for the *Hydra-Shok*, and 1.31 (0.33) cm^2 for the *Black Mamba*. The ANOVA test showed a significant difference between these three bullet types ($p = 0.002$). The *post-hoc* tests revealed a significant difference ($p = 0.006$) in the cross-sectional extent of the cavity volume between the *Hornady FTX* and the *Black Mamba*. The *Hornady FTX* and the *Hydra-Shok* showed outliers with values of 7.63 and 5.61 cm^2, respectively (Figure 4). After

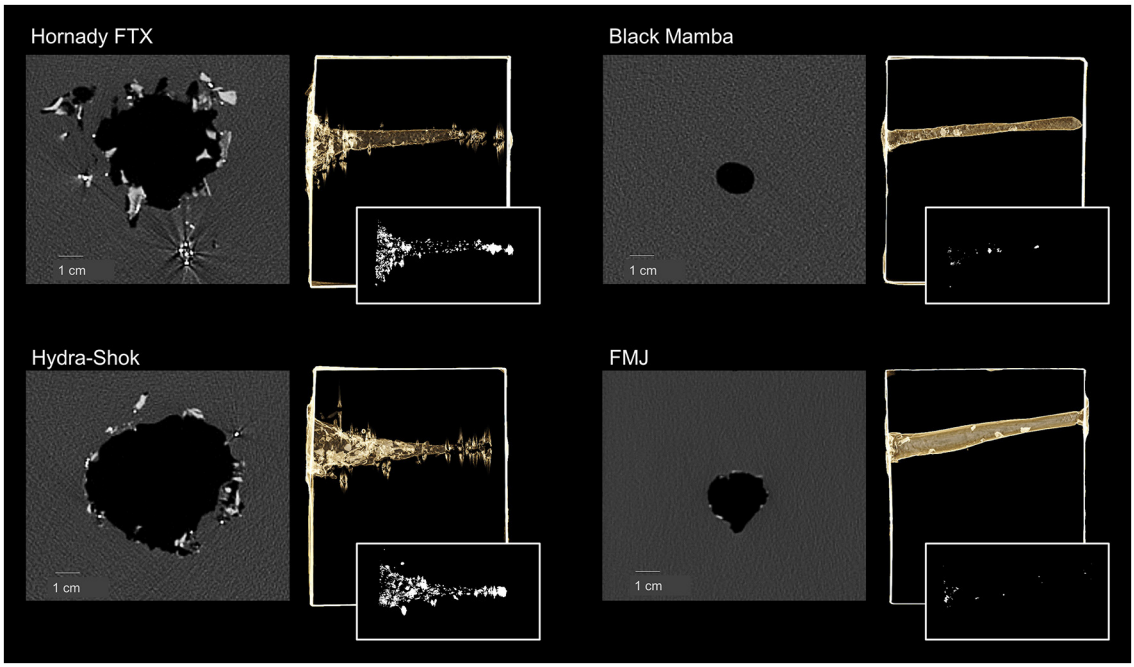


FIGURE 3
Transverse cross-sectional CT image through the cavity immediately after the perforation of the frontal bone sample and penetration into the soap block (for each bullet type, the left image in grayscale), volume rendering to show the entire cave in the soap block (for each bullet type, the right image kept in the color of the soap block), and maximum intensity projection to highlight bullet fragments along the cavity (for each bullet type, the right small image in black and white).

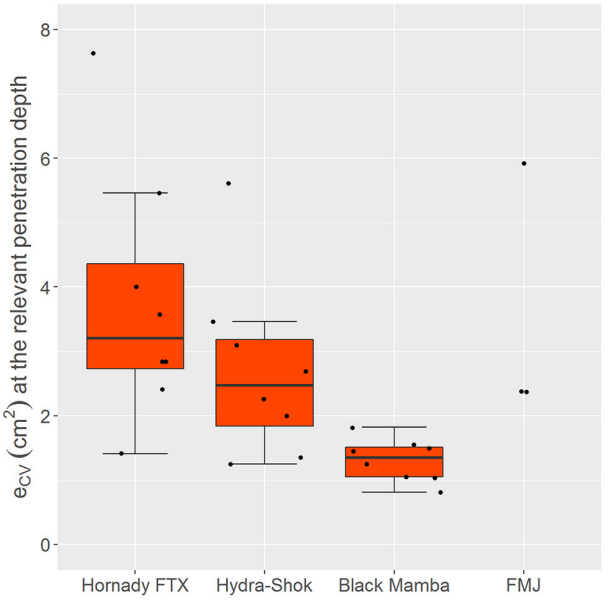


FIGURE 4
Boxplots of the mean cross-sectional extent of the cavity volume (e_{CV}) in cm^2 along the relevant section of the penetration depth into the soap block (5.5–7.5 cm). The mean volume extent of the *Hornady FTX* and the *Hydra-Shok* was larger than that of the *Black Mamba*. Outlier were observed for the *Hornady FTX* (7.63 cm^2) and the *Hydra-Shok* (5.61 cm^2). The *FMJ* achieved values comparable to the *Hydra-Shok*, assuming an outlier at 5.92 cm^2.

exclusion of the outlier, the mean volume extent was 3.22 (1.29) cm^2 for the *Hornady FTX* and 2.30 (0.84) cm^2 for the *Hydra-Shok*. For the *FMJ*, the cross-sectional extents of the cavity volume were 5.92, 2.37, and 2.38 cm^2.

Discussion

This study shows the difference between four bullet types when shot with the *BigBovid* through frontal bone plates of heavy cattle into ballistic soap, which allows for assessing the injury potential based on the cross-sectional extent of the cavity volume along the relevant section of penetration depth.

Both, the *Hornady FTX* and the *Hydra-Shok* demonstrated similar mean energy densities. This may seem astonishing at first glance, since the mass of the *Hydra-Shok* was only a factor of 1.13 heavier than the *Hornady FTX*, but the latter reached a velocity almost 30 m/s higher ($\Delta v = 29.81$ m/s), and the velocity is squared in the equation for the kinetic energy. In fact, the mean velocity of the *Hydra-Shok* was only a factor of 0.94 less than that of the *Hornady FTX*, and since the velocity is squared in the kinetic energy equation, the differences in mass and velocity equalize in the result, i.e., the mean kinetic energy. Regarding the *Black Mamba*, the low velocity together with the low mass resulted in significantly lower energy values compared to the other three bullet types. The results of the velocity measurements were similar to those of the previous study mentioned in the Section Introduction (3), in which the mean velocity was 499.89 m/s for the *Hornady FTX* and 384.79 m/s for the *Black Mamba*.

The energy density showed the same ratios between the bullet types as the kinetic energy due to the same reference areas, respectively, the same caliber. Consequently, due to the proportionality between energy density and penetration depth, the *Hornady FTX* and the *Hydra-Shok* are expected to penetrate more than twice as far into tissue, assuming that the trajectory of the projectiles is stable. A stable trajectory means that the bullets do not rotate, nor deform or fragment. However, especially due to fragmentation, the penetration depth can decrease considerably. This is mainly due to the strongly decreasing kinetic energy due to fragmentation. A portion of the kinetic energy is converted for the process of fragmentation and remaining kinetic energy is distributed to the individual fragments. From the results of this study, it is obvious that the *Hornady FTX* and the *Hydra-Shok* began to fragment as they penetrated the bone plate, while the other two bullet types were largely unfragmented. Therefore, a large proportion of the kinetic energy has likely been dissipated by the time the *Hornady FTX* and the *Hydra-Shok* penetrated the bone plate. As a result, these two bullet types did not pass through the soap block and eventually perforate the occipital bone plate behind it despite their high energy density, while the *FMJ* with a significantly lower energy density did so in every experiment. The question therefore arises as to the informative value of the energy density for determining the penetration depth through bone structures. In the end, the decisive factor is whether and how much energy was released at the relevant penetration depth, which can be inferred from the extent of the cavity volume.

Based on the extent of the cavity volumes at the relevant penetration depth of 5.5–7.5 cm in the soap block, the injury potential of the *Hornady FTX* and the *Hydra-Shok* is significantly and considerably greater than that of the *Black Mamba*, respectively. The *FMJ* in comparison showed comparable values to the *Hornady FTX* despite the narrow channel. This may seem surprising at first glance, since the extent of the cavity volume of the *Hornady FTX* and *Hydra-Shok* is very large due to fragmentation upon entry into the soap block, but decreases substantially with increasing penetration depth. Despite the comparable injury potential of the *FMJ*, the use of this type of bullet for the stunning of heavy cattle is strongly discouraged because over-penetration was observed in all experiments. In the previous study (3) where the *BigBovid* was used in regular slaughtering, over-penetration was observed even with the *Black Mamba*, although not so in the present study. Consequently, bullets that fragment but reach the relevant penetration depth are preferable for safety reasons.

Safety and proper use are important criteria for the use of a bullet shooting stunner such as the *BigBovid* and therefore a gun purchase license is required. In fact, already at the beginning of the twentieth century stunning devices for cattle have been developed, which discharged a bullet previously inserted into the barrel by striking the firing pin with a wooden hammer; but with these devices, accidents involving gunshot wounds to butchers soon became more frequent, so many butchers were already switching to the safer captive-bolt stunners (11). Today, such bullet shooting stunners are occasionally still in use to stun cattle. A study in the 1980s showed that the *Humane Killer* could fire a 10-g bullet

at a velocity of 165 m/s, corresponding to a kinetic energy of 136 J, which was considered sufficient for stunning cattle, based on preliminary studies that showed it would take about 127 J to penetrate the skull of a cattle (12). In this study, a 10-g bullet with 49 lead pellets in a polyethylene casing similar to a shotgun shell was used so that the bullet fragments and the individual lead pellets distribute in the brain, thus preventing over-penetration. Results recently published data (7) showed a kinetic energy of 164 J for an 8-g bullet with a caliber of 7.5 mm when use a *Humane Killer* resulting in an energy density of 3.71 J/mm^2 (note: in the study an energy density of 2.9 J/mm^2 is given, which is based on the kinetic energy divided by the caliber squared), which, was enough to pass through the bone plates of water buffaloes, but the bullet had only 8% of its initial kinetic energy left. In the case of heavy cattle, it can therefore be assumed that the *Humane Killer* does not provide the required energy density for the bullet to penetrate the thicker skull plates. Bullet shooting stunners recently developed for water buffaloes (13, 14) are capable of giving a bullet the required energy density, but to our knowledge these are only prototypes so far and are not commercially available. The *BigBovid* used in this study is commercially available and has already shown satisfactory results in the context of regular slaughtering of heavy cattle. In the end, however, it all comes down to the conscientious and practiced handling of the butcher. On the one hand, the butcher must be aware that the stunning device is firing a bullet and, on the other hand, the bullet shooting stunner must be placed at the correct position and operated at the correct angle so that the relevant brain regions are appropriately injured in the process of creating the temporary wound cavity.

In the present study, the cavity volume determined on the CT data was not related to the transferred energy. Although the proportionality factor between cavity volume and energy transferred to the medium is considered to be independent of the shape and size of a metallic projectile when the transferred energy is <2,500 J (10), it is not described how this proportionality between cavity volume and transferred energy behaves when a bullet fragments along its path through the medium. Since both the *Hornady FTX* and *Hydra-Shok* were highly fragmented in the present study, no conversion of the resulting cavity volume to the transferred energy was carried out.

This study has some limitations. First, it should be pointed out that a homogeneous soft tissue simulant was used, which represents tissue very well, but is not identical in its material properties. Although the experimental data of this study are in agreement with the data on the use of the *BigBovid* in the earlier applied study, deviations in routine application cannot be excluded. Second, there are discrepancies between experimental setting and anatomical conditions. Thus, the brain tissue is enclosed by the bony skull and is also smaller in volume compared to the soap block. These discrepancies were considered negligible for the purpose of comparing the four bullet types in terms of their injury potential. Third, the distance between the muzzle of the bullet shooting stunner and the object may show variations in the results. In the event of a contact shot, the propellant gas can penetrate the tissue, contributing to its destruction. At a distance of a few centimeters, this additional effect is lost. In this study, the *BigBovid* was applied directly to the removed forehead plates. However, since the soap block was not fully surrounded by bony structure, the effect of propellant gases may be reduced. Fourth, metal artifacts of the larger fragments on the CT images might influence the segmented cavity volume. However, these deviations in the exact determination of individual volumes are considered negligible in view of the differences in volumes in individual experiments for one and the same bullet type. The method of segmentation itself instead of measuring directly on the soap block is considered accurate. A recent study showed very high accuracy for CT-based determinations of the cavity volume compared to a common method using silicone castings, which turned out to be far less accurate (15).

Conclusion

Based on the results of this study, we recommend two bullet types for stunning heavy cattle with the *BigBovid*, namely the *Hornady FTX* and the *Hydra-Shok*. First, these bullet types have a high energy density and thus a high penetration potential to pass through the thick frontal bones of heavy cattle. Second, over-penetration is unlikely due to the high fragmentation of the *Hornady FTX* and the *Hydra-Shok*. Third, at the relevant penetration depth, these two bullet types caused extents of the cavity volumes equivalent to and greater than those of the *FMJ* and *Black Mamba*, respectively, and therefore the injury potential of the *Hornady FTX* and *Hydra-Shok* is considered adequate for stunning heavy cattle. Approval of the *BigBovid* with the *Hornady FTX* and *Hydra-Shok* for stunning heavy cattle can now form the basis for further large-scale field studies.

Data availability statement

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

Ethics statement

Ethical review and approval was not required for the animal study because no study was performed on live animals. However, we would like to mention here that frontal bone plates of heavy cattle, taken after regular slaughtering, were used for the experiments. No animal was killed for the purpose of the study.

Author contributions

DG: conceptualization, investigation, methodology, formal analysis, writing and editing, and visualization. RS and NZ: review and editing. MV: investigation. MO: visualization. MT: resources. HR: project administration, conceptualization, investigation, review, and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

MV was employed by the company Vogt Waffen AG.

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2023.1143744/full#supplementary-material

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A century of "Camel Research": a bibliometric analysis

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Introduction: Bibliometrics is a quantitative analytic strategy used to assess the unit of publications per each field of research. Bibliometric studies are commonly employed to examine the current research climate, potential developments, and development trends in certain domains. In this work, the major contributors to camel research throughout the past century are discussed, along with the funding sources, academic institutions, scientific disciplines, and countries that contributed to "Camel Research".

Methods: The Web of Science (WOS) database was used to retrieve the publications based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) instructions.

Results: There are 7,593 articles dedicated to camel research on the Web of Science (as of August 1st, 2022). Three stages were involved in the publication of a study on camels. At the beginning, from 1877 to 1965, there were fewer than ten new publications per year. The second stage comprised 100 publications per year (1968–2005). Since 2010, nearly 200 new papers have been published each year. King Saud and King Faisal universities contributed > (0.08) of the total publications. While more than 1,000 funding agents were retrieved, the Natural Science Foundation of China (NSFC) showed the greatest rate of funded projects (0.17). Camel research was included in 238 scientific disciplines. The top disciplines were Veterinary Sciences (0.39), Agriculture Dairy Animal Science (0.144), and Food Science Technology (0.087).

Conclusion: There has been an increase in interest in camels in recent years, but the research trends in camel health and production need greater support.

KEYWORDS

bibliometrics, camels, web of science, camel, research

1. Introduction

To assess the present state of research in a field, bibliometric techniques are utilized. The bibliometric analysis makes it much simpler to determine, within a specific area of research, the research scholars, universities, papers, and search terms that receive the most attention and are most frequently cited. This is possible through the identification of the most proactive and highly cited research (1). Additionally, it allows experts to evaluate a publication's quality and coherence, and it can also manifest the author's profile. Anticipating emerging trends in different fields is one of the key benefits of this type of study (2). It is frequently used to investigate the state of existing research, future perspectives, and growth patterns of certain fields (3). Publications are one of the key means by which advances in scientific research and technological development are displayed. The extrinsic parameters of scientific publications serve as the research subjects for the quantitative analysis method known as bibliometrics (4). As per various illustrations of experimental observations, this approach can be classified into bibliographical statistical, mathematical, system, matrix and network analysis (1). Thus, the current study uses bibliometric analysis to follow the development of camel research and its effects on academia and the public across published works that use camel referencing papers included in the Web of Science (WOS) (1900–2022).

The camel is considered the most suitable domesticated animal for desert regions with sporadic and inconsistent yearly rainfall and lengthy, arid, warm seasons lasting at least 8 months (5). Camels have been domesticated for approximately 3,000-6,000 years, Old World camels have benefited humans in cross-continental migrations by carrying people and commodities, bridging cultural barriers, and producing milk, meat, and wool (6). Animal husbandry and livestock science are challenged by the expansion in deserts caused by extreme weather events and the rising demands for sustainably produced meat and dairy products (7). The dromedary camel should be taken into consideration as the most adapted and sustainable organism that could be used the harsh climate caused by global changing weather patterns that are defined by a consistent rise in desertification, high temperatures, and also water shortages. Dromedary camels are adaptable to these extreme weather changes and extremely effective in their production (8, 9). Interestingly, several recent reports have shown that the dromedary camel would be the foremost species that manage to survive as the best source of farm animals for future agribusiness and the animal production industry thanks to the advances of the desert world, primarily to actively participating in achieving the Sustainable Development Goals (9–12). Dromedaries, however, have gained attention from the research community than other farmed animals (13). In this regard, a greater awareness of the number of camel-related research studies that have been conducted and the leading camel research nations will assist to enhance the sort of research that should be conducted in the future.

Previously, very limited bibliometric studies have been conducted on camels (14, 15). One of these bibliometric studies investigates 4,923 camel scholarly articles from 1963 to 2012 (a period of 50 years), which were retrieved from the CAB Direct Online database (14). Recent reports have also traced past scientific papers indexed in the ScienceDirect directory and analyzed the impact on camel husbandry and welfare (15).

A systematic approach for tracking the advancements in an area of study and identifying the attributes of certain publications is offered by bibliometric analysis. This article offers a bibliometric study of camel-related scientific publications from 1877 to 2022. This will help in examining the state-of-the-art research products in the camel sector, including key discipline statistics, famous journal developments, and publication patterns in literature. Moreover, to take into account the representative regions, prominent authors, and most referenced publications.

2. Materials and methods

2.1. Theoretical aspects of bibliometric method

Bibliometrics is the study of quantitative methods and measures for analyzing and evaluating scholarly publications and communication. It is an interdisciplinary field that draws on information science, mathematics, statistics, and sociology. The main objective of bibliometrics is to provide objective, quantitative measures of the impact and influence of research publications, authors, and journals. The development of bibliographic databases, such as the Science Citation Index, Web of Science, Scopus and others, made it possible to analyze the patterns of citation and publication in scientific literature. Bibliometrics uses a variety of quantitative measures to assess the impact and influence of scholarly publications, such as publications count, active researchers and institutions. Bibliometric analysis is widely used in academia, research institutions, and funding agencies to assess the impact and influence of research publications and to make decisions about funding, promotions, and tenure.

2.2. Database search and retrieval of articles

The data collection was achieved by first identifying the databases and choosing appropriate search strategy techniques, data retrieval techniques, and cleaning the data before feeding them into different tools for analysis and visualization. For this research, WOS was used to retrieve the bibliometric data, and the data was exported to Excel. The study used Excel to provide the visual graphs for the bibliometric data on camels.

The term "camel" that was included in the title, abstract, and keywords was used to retrieve bibliographic data from the WOS database. The WOS database is the primary data source for bibliometric studies and offers extensive, multidisciplinary citation data. A large volume of data was retrieved for the years 1877 to 2022. Using bibliometric principles, the categories assessed were affiliations, authors, document type, funding, countries/regions, grant numbers, open access, publication year and WOS category. These are the major bibliometric parameters established in other research publications (16). Bibliometric analysis can be used to analyze a research trend in a particular field.

2.3. Eligibility assessment

Two independent reviewers assessed the eligibility of the studies included in the bibliographic analysis based on several

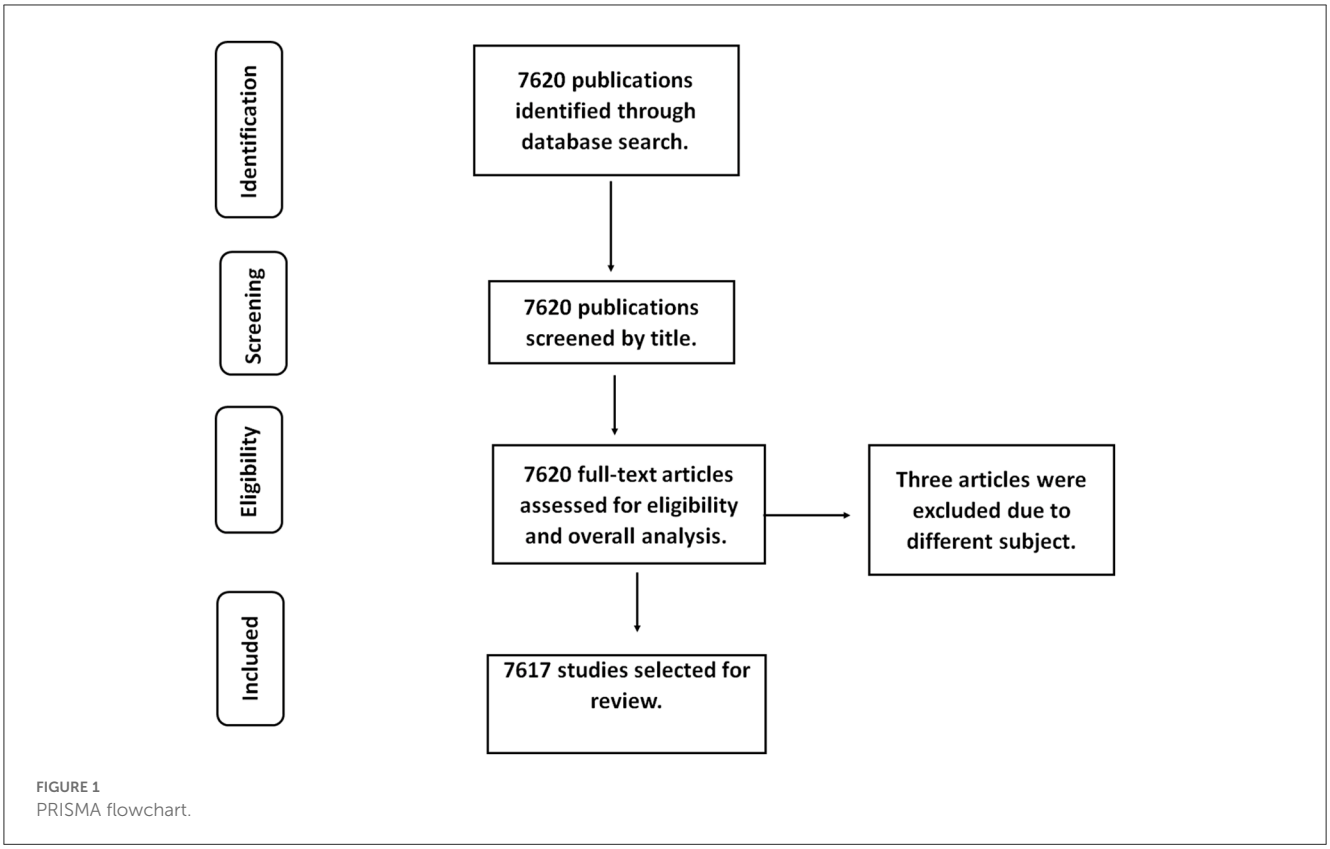

FIGURE 1
PRISMA flowchart.

criteria. Foremost, the study restricted the literature to those only published in English. The articles selected were first based on the relevant content provided in the title and the abstract. The exclusion criteria also involved those studies that were not using camels in the experiments. During the review, critical features extracted from the full text included the author, titles, recent citations, year of publications, institutions of research and funding agencies. Finally, any disagreement arising from the inclusion or exclusion of a study was resolved through consensus. The PRISMA flowchart for the article retrieval steps is provided in Figure 1.

2.4. Article inclusion/exclusion criteria

The study did not exclude any of the parameters provided in the WOS since the study intended to explore all the publications stored on camels. Retrieved documents included articles, book chapters, editorial materials, and reviews. Since only English words were employed to search for relevant publications, the search may have left out some critical articles published in other languages such as French, Spanish, and Chinese. Therefore, the results may not be generalized to other researchers published in non-English speaking countries. Although the analysis may not include all the crucial publications on camels, the researcher believes that this study's results offer a reliable insight into the trends and patterns of the publications made on camels.

3. Results and discussion

Using the term "camel" in the paper title, summary, or search terms, the WOS database was accessed for collecting reference information on the study subject. A total of 7,617 documents were extracted from WOS between the period 1877 and 2022. The information that was acquired provides insights into the most prominent scholars and the most active institutions in the field of camel research, which may also be helpful for the identification of funding opportunities and potential for collaboration.

3.1. Document types

The type of document retrieved was analyzed based on the WOS database classification. Publications on camels were distributed in 26 publication types. Out of a total of 7,617 documents, 6,052 (0.794) documents were articles which indicate article was the most prevalent type of document and has the highest contribution to camel research during the 1877–2022 period. Furthermore, proceeding papers (312; 0.041), meeting abstracts (293; 0.038), notes (244; 0.032), book chapters (216; 0.028), book reviews (207; 0.027), Editorial material (158; 0.07), review article (145; 0.019), letter (124; 0.016), corrections (44; 0.006), news items (41; 0.00538), poetry (35; 0.00459), early access (24; 0.003), fiction, creative prose (13; 0.0017), film reviews (6; 0.00079), record reviews (5; 0.00066), books (4; 0.00053), data papers (4; 0.00053), correction, addition (3; 0.00039), excerpts (3; 0.00039), art exhibit

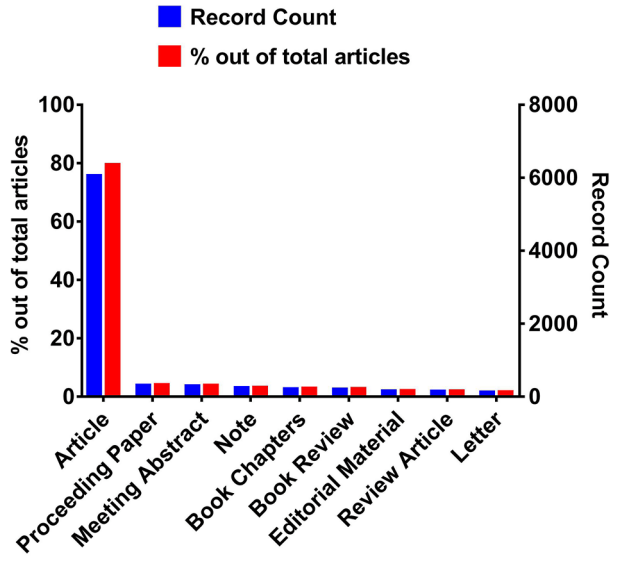

FIGURE 2
The number of document types published between 1900–2022 in the camel research field in WOS.

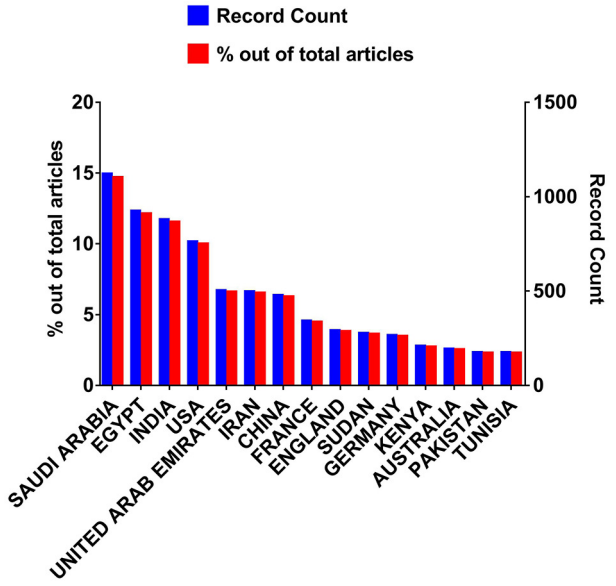

FIGURE 3
The top fifteen nations that published the most papers on camels between 1877 and 2022.

reviews (2; 0.00026), reprints (2; 0.00026) type documents were also found. While biographical-items, dance performance reviews, hardware reviews, and software reviews were the type of documents that showed the lowest contribution (1, 0.00013) in camel research during the 1900–2022 period. Figure 2 represents the document types out of a total of 7,617 articles contributed in the camel field between 1877–2022 time interval. The observed number of open-access papers in camel research highlights the recent trends in funding agents of camel research projects to spread camel-related knowledge. The roles played by funding agencies and research organizations in the advancement of scientific inquiry are of the utmost significance (17, 18).

3.2. Countries/regions

Regarding the countries, a total of 7,617 articles were published in 131 countries according to the literature database. Figure 3 shows the top 15 countries with a maximum number of publications during 1877–2022. The complete list is provided in Supplementary Table 1. These countries include Saudi Arabia, Egypt, India, USA, United Arab Emirates, Iran, China, France, England, Sudan, Germany, Kenya, Australia, Pakistan and Tunisia. Saudi Arabia appeared as the country with the highest number of publications i.e., 1,117 (0.1467) papers out of 7,617 published papers. This showed the highest contribution of Saudi Arabia in the field of camel research. Egypt ranked second with 921 (0.120) papers out of 7,617 published papers followed by India which scored in the third position due to the publication of 876 (0.115). Thus, Saudi Arabia, Egypt, and India are the top three countries with the highest number of publications related to the camel field and playing a major role in this field. The USA with 759 (0.0997) publications out of 7,617 papers stand at the fourth position, UAE at the fifth position with 500 (0.066) publications, Iran at sixth position with 494 (0.0649) publications, China at seventh position with 474 (0.06223) publications, France at eighth position with 338 (0.0444) publications, England at ninth position with 288 (0.0378) publications, Sudan at tenth position with 288 (0.0378) publications, Germany at 11th position with 262 (0.0344) publications and Kenya at 12th position with 205 (0.0269) out of 7,617 papers. While publications from Australia, Pakistan, Tunisia, Morocco, Italy, Ethiopia, Spain, Japan and Canada countries range between 100–200 i.e., 190 (0.0249), 172 (0.0225), 172 (0.0226), 153 (0.0201), 145 (0.019), 132 (0.0173), 110 (0.0144), 107 (0.014), 105 (0.0138) respectively. Moreover, Sweden, Algeria, Jordan, Belgium, Nigeria, Turkey, Oman, Kazakhstan, Netherlands, Switzerland, Austria, and Russia with publications ranging between 50–99 papers from 1900 to 2022 i.e., 98 (0.01287), 95 (0.0125), 92 (0.012), 84 (0.011), 83 (0.0109), 78 (0.01024), 77 (0.0011), 76 (0.00998), 70 (0.00919), 65 (0.00853), 54 (0.0071) and 52 (0.00683) papers, respectively. Denmark, Scotland, Iraq, Malaysia, Mongolia, Qatar, Taiwan, Kuwait, South, Africa, Fed Rep Ger, Libya, Hungary, Brazil, Ireland, Czech Republic, Somalia, South Korea, New Zealand, Poland, Norway, Syria, Thailand, Uganda, Botswana, Singapore, Mauritania, Uzbekistan published 10–49 papers during the period of 1900-2022 such as 47 (0.00617), 43 (0.00565), 41 (0.00538), 41 (0.00538), 39 (0.00512), 39 (0.00512), 38 (0.00499), 36 (0.00473), 35 (0.00459), 28 (0.00368), 27 (0.00354), 26 (0.00341), 24 (0.00315), 24 (0.00315), 23(0.003), 23 (0.003), 22 (0.0029), 20 (0.0026), 17 (0.0022), 16 (0.0021), 16 (0.0021), 14 (0.0018), 13 (0.0017), 12 (0.0016), 12 (0.0016), 11 (0.0014) and 10 (0.0013) papers respectively. Argentina and Bahrain both published nine (0.0012) papers, each of three countries i.e., Greece, Portugal and Romania published eight (0.0011) papers, each of four countries i.e., Ger Dem Rep, Lebanon, United Arab Rep, Yemen published seven (0.00092) papers, each of four countries i.e., Bangladesh, Serbia, Turkmenistan and Ussr published six

(0.00079) papers, each of six countries i.e., Burkina Faso, Mali, Niger, Tanzania, Vietnam and Wales published five (0.0007) papers from 1900 through 2022. Chad, Croatia, Indonesia, Malawi, and Senegal were the countries that published four (0.0005) papers, while Chile, Ecuador, Eritrea, Mexico, and Slovenia each published three papers from 1900 through 2022. Each Azerbaijan, Bulgaria, Cambodia, Czechoslovakia, Djibouti, Ghana, Jamaica, Lithuania, Namibia, North Ireland, and Peru published only two papers related to the camel field during the 1900–2022 time period. Each of 29 countries including Afghanistan, Bosnia Herceg, Brunei, Burundi, Colombia, Costa Rica, Cuba, Dem Rep Congo, Honduras, Hong Kong, Kosovo, Latvia, Liberia, Luxembourg, Madagascar, Monaco, Mozambique, Nepal, North Korea, Palestine, Rep Congo, Slovakia, Sri Lanka, Trinidad Tobago, Uruguay, Venezuela, West Germany, Yemen Arab Rep, Yugoslavia, Zambia were the countries which published least number papers i.e., only one (0.00013) during the 1900–2022 period.

The observed findings coincide with the previous reports of camel livestock contribution by countries. Saudi Arabia has been classified as one of the countries with a high proportion of camel livestock (19). Egypt showed second rank in the list of publications, and despite Egypt's classification as a camel country, it has a declining growth with a low proportion of camel livestock (19). There is an increasing interest in camels in China in recent years. Individuals in China are becoming more interested in drinking camel milk because of the purported health benefits it offers as well as the wool and leather industry from Bactrian camels (20–22). In the USA, there are only 3,000 heads of camels distributed among US private farms (19). There is now increasing interest in camels due to the beneficial use of camel milk in treating diabetes, colitis and other somatic diseases (23). The United Arab Emirates is classified as one of the countries with an extremely lofty proportion of camel livestock (19).

3.3. Authors

Results from WOS illustrated that Faye B was the most productive author with 151 (0.0012) publications in the camel research field from 1877 to 2022 followed by Wernery U (95; 0.0125), Wang JL (84; 0.011), and Gahlot TK (72; 0.0095). So, these were the authors who contributed most to the camel research field. Figure 4 shows the top 20 authors with the highest number of publications searched from WOS. The complete list of authors and their contributions is provided in Supplementary Table 2.

3.4. Affiliations

Affiliation of most of the publications found with Egyptian Knowledge Bank (EKB) (824; 0.1082), followed by King Saud University (416; 0.055), King Faisal University (320; 0.042), Indian Council of Agricultural Research (306; 0.04), National Research Centre on Camel (261; 0.034), Rajasthan University of Veterinary Animal Sciences (211; 0.0277), Cairo University (201; 0.0264), University of Khartoum (187; 0.0256), United Arab Emirates University (165; 0.0217), and CCS Haryana

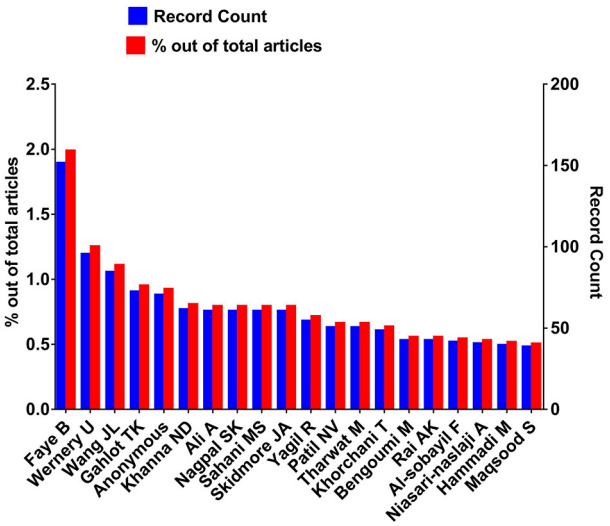

FIGURE 4
Top twenty most active camel researchers who published between 1877 and 2022.

Agricultural University (134; 0.0178). This data shows that most of the studies were conducted by authors affiliated with Saudi Arabia and Indian Universities. Figure 5 shows the most common affiliations of camel-related publications. The complete list of affiliations and their contribution to camel research is provided in Supplementary Table 3. This data is in line with the previous classification of Saudi Arabia as a country with a sharp increase in camel population following regular growth (19). This could be accompanied by increased interest in developing camel research. According to the data that is curated by the Ministry of Environment, Water, and Agriculture in Saudi Arabia, the number of camels is estimated to have more than 1.39 million heads in 2018 and has been growing annually by 0.52 since 1961. This represents a significant increase from the number of camels that existed in 1961 (24). Camel countries with a sharp increase in camel population following regular growth comprise countries within the top common affiliations.

3.5. Trends in scientific publications (Per-year research)

This investigation displays camel-related research publications from 1900 through 2022 in order of publication year. Article counts by year, from 1900 through 2022, are shown in Figure 6. From the graph, we may deduce that between 1900 and 1960, annual publishing rates were rather low, falling in the range of 1–6. Annual publishing rates rose steadily from 1961 to 1999. There were over a hundred publications that were seen for the very first time in 1993 (115; 0.015). More than a hundred articles were published between 1994 and 1996. More than 100 papers each year have been made in camel research from the year 2000 until 2022. In 2020, there were 433 papers published in camel research, representing

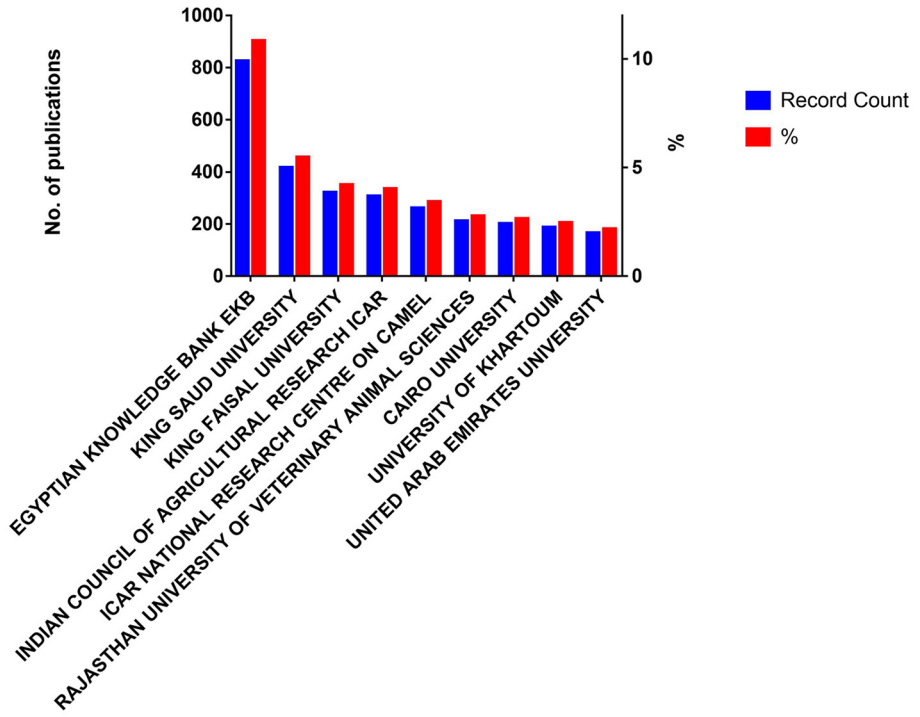

FIGURE 5
Most common affiliations of camel-related publications during 1877–2022.

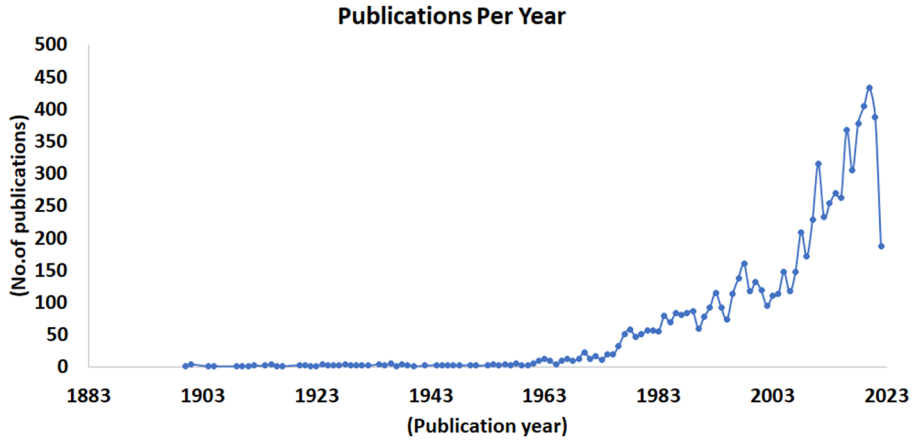

FIGURE 6
Number of articles per year in camel research published during 1877–2022.

a 5.68 percent annual increase over the previous year's output. Annual publishing rates fell to 388 (0.051) in 2021. There will have been 187 papers published by August 2022, representing 0.0246 of the total. This demonstrates that 2020 was the most fruitful year for camel study. It's possible that one of the reasons for the recent uptick in camel research is that (1) there are now more camel-focused publications and special issues available. (2) In recent years, the association of camels with respiratory virus outbreaks known as MERS-CoV infection has contributed to an increase in the number of camel studies and citations for those studies. Among these are works that are relevant to MERS-CoV that have received a large number of citations in the camel scientific community. For instance, highly cited articles included 975 (25) and 411 (26) citations.

3.6. The most active funding agents

Considering funding, out of 7,617, a total of 2,078 publications appeared in the search through WOS. The highest publications i.e., 137 (0.0179) received funding from the National Natural Science Foundation of China (NSFC) followed by King Saud University

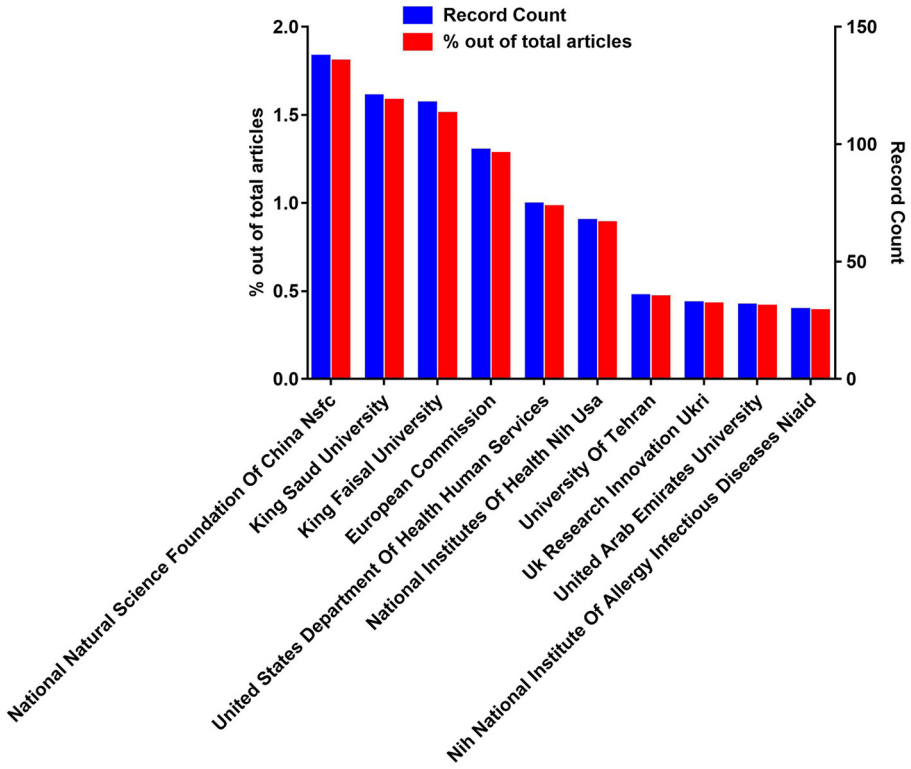

FIGURE 7
The top ten most active funding agencies in camel research.

(120, 0.0158), European Commission (97; 0.0173), United States Department of Health and Human Services (74; 0.0097), and National Institutes of Health (NIH) USA (67; 0.0088). While the rest of the funding agencies provide funds to <10 publications. This shows that NSFC is one of the main funding agencies with a significant contribution to accelerating the research related to camels. Figure 7 shows the top ten funding agencies empowering camel research. The complete list of funding agents is provided in Supplementary Table 4.

3.7. Grant number and number of publications

Grant numbers are unique identifiers assigned to research grants by funding agencies or institutions. In bibliometric analysis, grant numbers are important because they allow researchers to track the impact of funding on scientific publications. By analyzing grant numbers, bibliometricians can identify the research projects that were funded by a particular grant or funding agency. This information can then be used to evaluate the productivity and impact of the research produced by the grant recipients. Grant numbers can also be used to identify collaborations between different institutions or researchers, as well as to identify trends in research funding over time. In addition, grant numbers can be used to identify potential conflicts of interest in research. For example, if a particular grant is awarded to a researcher who is also a member of a company's board of directors; this could raise questions about the objectivity of the research findings.

From 1877 to 2002, WOS databases turned up 1,530 grant numbers associated with camel research. I.b/1.1/493 and Upar-31f09417 grant numbers were mentioned by the highest number of publications (17; 0.233), while 2015dfr30680 and Ky201401002 grant numbers were mentioned in 14 (0.00184) publications individually. 31360397 (13; 0.0017), Utf/sau/021/sau (13; 0.0017), 2008sklab06-05 (12; 0.0016), 223498 (12; 0.00156), 2018bs03017 (11; 0.0014), 39300097 (11; 0.0014), Ndyb2017-28 (11; 0.0014), Rg-1438-018 (11; 0.0014) and P29623-b25 (10; 0.0013) were observed in more than 10 publications searched through WOS. The rest of the grant numbers were found in fewer publications. Figure 8 illustrates the top thirty Grant Numbers related to camel research.

3.8. Open access

Open access is very important in bibliometric analysis because it provides researchers with unrestricted access to scientific literature, which allows them to discover and use a wider range of sources in their analysis. Open-access articles are available to anyone with an internet connection, which can increase the visibility and impact of scientific research. Open access is a critical component of bibliometric analysis because it promotes greater scientific discovery and collaboration, while also enabling more accurate and comprehensive analysis of scientific literature. By making research articles freely available, open access promotes greater collaboration and exchange of ideas among researchers, which can lead to new discoveries and advances in science. It also enables research to be more easily reproduced and verified,

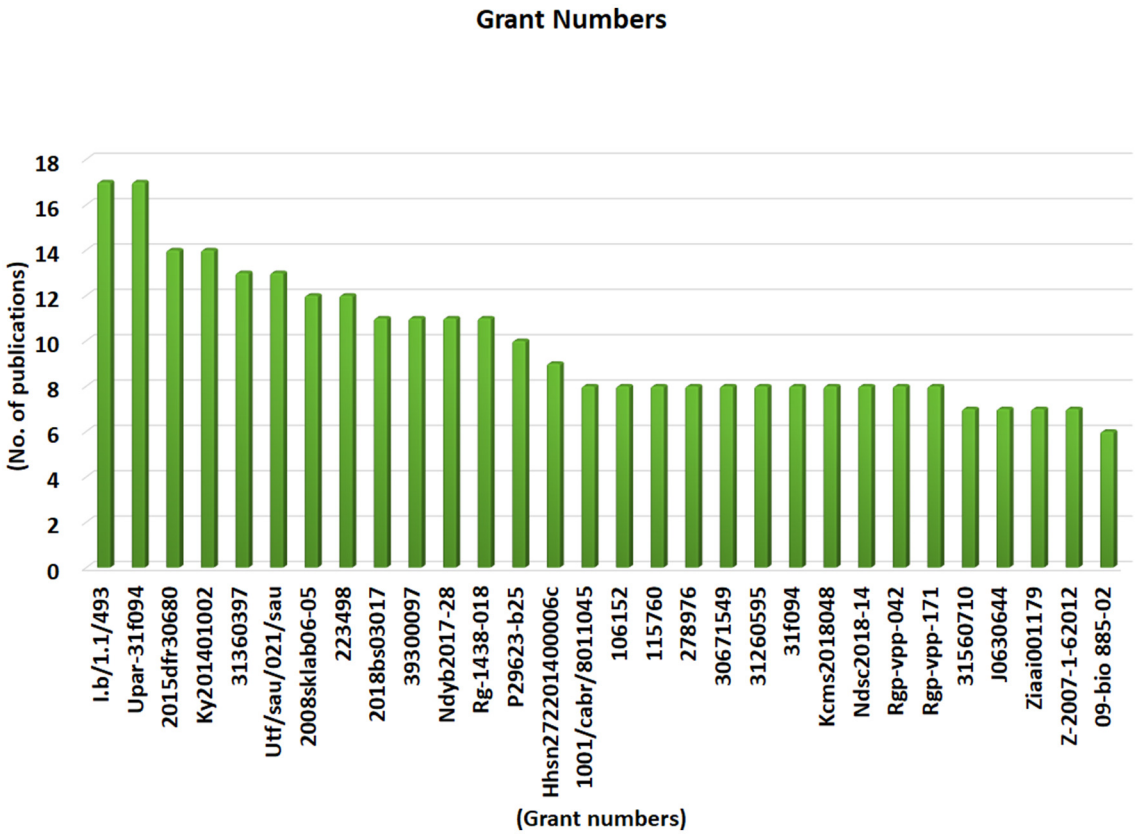

FIGURE 8
Top thirty Grant Numbers related to camel research searched from WOS between 1877–2022.

which is an important aspect of scientific inquiry. In bibliometric analysis, open access can facilitate the identification of relevant publications for citation analysis and impact measurement. It can also reduce bias in the analysis by ensuring that all publications, regardless of their source or publisher, are available for evaluation. In this study, the fraction and distribution of open-access categories were analyzed.

From the total of 7,617 papers in the camel research field during the 1877-2022 period, 1,550 (0.2035) papers were published as all/complete open access, 886 (0.1163) with gold open access, 149 (0.01956) with gold-hybrid open access, 253 (0.0332) were free to read, 876 (0.115) were green published, 73 (0.0096) were green accepted and 395 (0.0519) were green submitted. So, most of the articles i.e., 1,550 in the camel research field during the 1877-2022 period were published with all open access as shown in Figure 9. The rest of the documents including 6,067 (0.7971) papers were not found in the open access category.

3.9. WOS categories

WOS stands for Web of Science, which is a bibliographic database that indexes and provides access to scholarly literature from various fields. WOS categories refer to the subject categories or fields in which the indexed literature is classified. The importance of WOS categories lies in their ability to organize and retrieve literature based on subject matter. Researchers and academics can use these categories to search for literature relevant to their area of study, as well as to identify research trends and collaborations within specific fields. However, there are also limitations to WOS categories. For example, some articles may fall into multiple categories, making it difficult to classify them accurately. Additionally, the categories themselves may not always accurately reflect the interdisciplinary nature of modern research, leading to potential biases in how research is organized and evaluated. Furthermore, not all academic disciplines are represented equally in the WOS database, which may limit its usefulness in certain areas of study.

All 7,617 publications related to camel search were observed in 238 categories of WOS. Of which the highest i.e., 3,023 (0.397) publications were collected from Veterinary Sciences followed by Agriculture Dairy Animal Science (1,097; 0.144), Food Science Technology (665; 0.0873), Zoology (408; 0.0536), and Biochemistry Molecular Biology (380; 0.0499). The top twenty WOS categories are shown in Figure 10. The complete list of WOS categories is provided in Supplementary Table 5.

The bibliometric research on camels is helpful, in particular to the veterinary practitioners as well as the academics, because it provides direction on the developments that are taking place in the sector. By browsing WOS categories of camel publications, camel practitioners can gain insight into the scientific disciplines and new discoveries in their field. Both veterinary practitioners and academics in several ways: 1) Identifying research trends: This

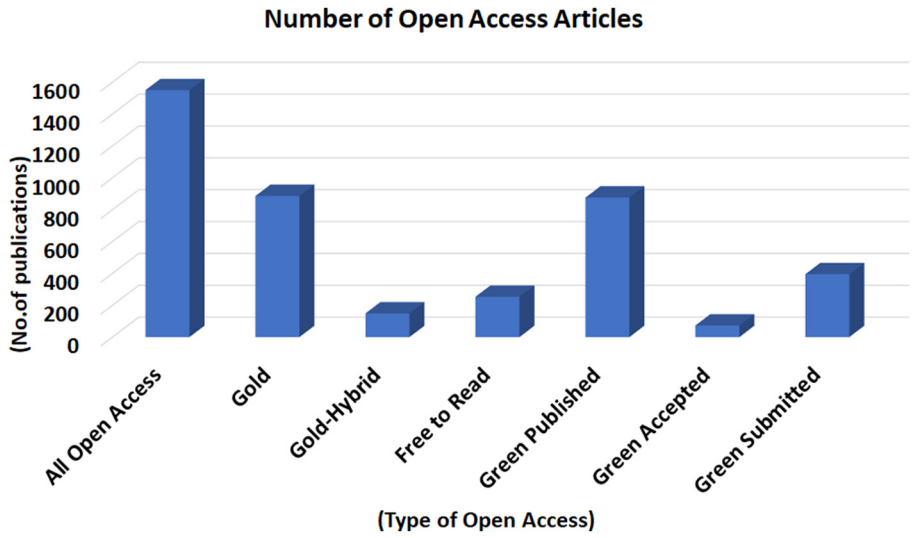

FIGURE 9
All Open Access publications in camel research during 1877–2022.

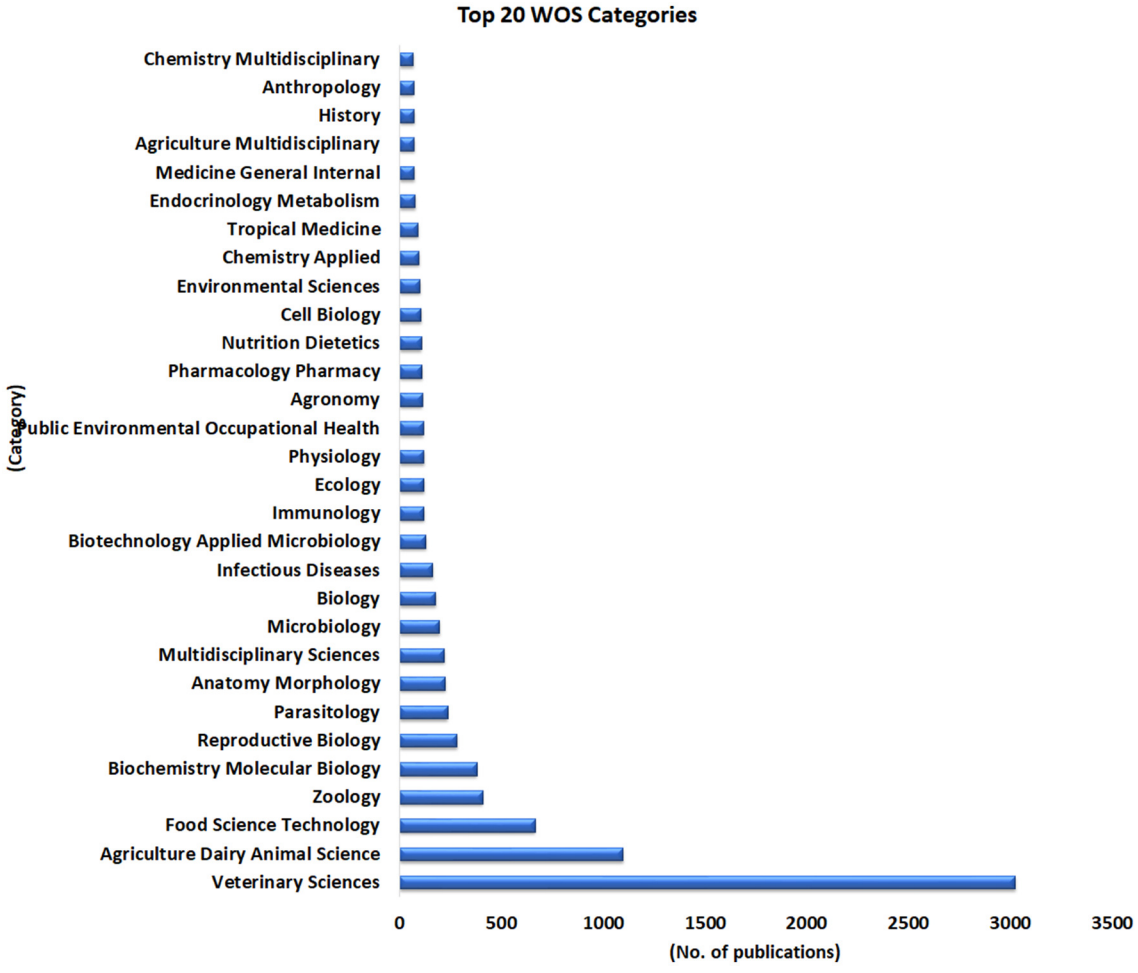

FIGURE 10
Top twenty categories of WOS with the highest number of publications in camel research during 1877–2022.

information can be used by veterinary practitioners and academics to focus their research efforts on areas that are receiving significant attention in the field. 2) Assessing the impact of research: By analyzing citation patterns, researchers can identify which studies have had the most significant influence on the field, and which have been less impactful. 3) Identifying knowledge gaps: Bibliometric analysis can also help identify areas where there is a lack of research. In addition, students can increase their knowledge of the right locations in which to seek the material that is important to the topic. Along with other approaches for the scientific visualization of mapping, the given co-author network may be able to provide insight into some of the authors who are particularly well-known in the area. In addition, there are other databases that can provide further information, such as the keywords that are used the most, referrals to publications, and citation networks. Because of this, one of the shortcomings of this study is that it only uses a single database. Nevertheless, there is a possibility that integrating many databases would provide skewed results due to the fact that part of the information may be readily replicated. On the other hand, upcoming scholars in this subject will be able to conduct analysis by retrieving data from other databases, such as Web of Science and Scopus. To further broaden the scope of bibliometrics in this area, future scholars might additionally assess the nations or institutions that have produced the most significant number of articles.

3.10. Limitations

A limitation of this study is that only articles that were published on the Web of Science were taken into consideration for this study. This is done to guarantee that the camel research that is published is of high quality. This might be considered a restriction as well because many additional papers are published in journals that are not included in WOS. The publications included in this work were only available in the English language, which is another limitation of this study.

4. Conclusions

This study used the bibliometric approach to analyze the patterns and characteristics of scientific literature in camel research, such as the number of publications, authors, journals, funding agents and scientific disciplines. Camel research studies revealed a dramatic rise in activity, funding, and participation from a wide range of countries. More scholarly publications pertaining to camels were published in 2020, roughly exceeding 200 publications per year, showing a rise in the field's profile. Because of this, researchers can now explore several previously unexplored facets of camels, including improved methods for the diagnosis, management, and prevention of infectious disease; increased milk and meat production; and, most importantly, the biological and pharmaceutical applications of camel milk. This study identified the most influential authors and institutions in camel research, which is led by several Asian and African countries. The study shed light and identified emerging research areas through the exploration of the WOS categories. There are already 238 disciplines that make use of camel research. Veterinarian medicine, dairy animal science in agriculture, and food science technology were the top three majors. Research into camel health and productivity is still needed, despite the animal's rising popularity in recent years. Continuous follow-up bibliometric studies are required to ascertain the newly added disciplines and patterns of camel-related publications.

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

MK, MMo, HA, MMa, WES, and HE-B planned the study design, contributed to data analysis, and wrote and revised the manuscript. MK, IA, and KV contributed to data extraction and analysis. All the authors approved the final version of the 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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2023.1157667/full#supplementary-material

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Functional measurement of canine muscular fitness: refinement and reliability of the Penn Vet Working Dog Center Sprint Test

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Working, sporting, and companion dogs require muscular fitness to perform their daily tasks, competitive activities, and operational functions effectively and with a low risk of injury. There are currently no methods to measure the muscular fitness of dogs who are not debilitated. Sprint performance is highly correlated with muscular fitness in humans, and various sprint assessments are used to measure performance for sporting and tactical athletes. The Penn Vet Working Dog Center Sprint Test (ST) is a 25 m maximal effort sprint from a down position and was developed to be a low-cost measure of muscular fitness suitable for field use. The purpose of this project was to describe the refinements to the ST, detail the performance and measurement protocol, evaluate the measurement inter-rater and intra-rater reliability, characterize the acceleration profile, and explore the inter-day reliability. Both naïve and experienced raters demonstrated excellent intra-rater and inter-rater reliability. The acceleration profile of the dogs in this study was similar to that of average adult human sprinters and demonstrated the role of muscular fitness in performance over this short distance. Finally, a small group of dogs showed moderate inter-day reliability and provided initial performance data to inform future studies. The ST appears to be a reliable measure of canine muscular fitness and could be used to assess performance in healthy dogs and guide the return to sport or work of debilitated dogs.

KEYWORDS

dog, working, sporting, fitness, sprint, test, rehabilitation

Introduction

All dogs should be physically fit enough to perform their daily activities and those supporting their relationships with other dogs and people (e.g., walks and playing). Some dogs perform more physically demanding tasks while training for or competing in sports or while training for or performing operational tasks like detection or apprehension. Dogs lacking the physical fitness required for their tasks risk diminishing their ability to perform them or becoming injured while performing them. Compared to pet dogs, fitness is of even greater

importance for working dogs as insufficient fitness may result in failing a task and subsequent injury to their human (and animal) teammates or another human (1–3).

Companion dogs, sporting dogs, and working dogs need well-rounded fitness, including the modalities of strength, power, mobility, cardiorespiratory endurance, balance, muscular endurance, and agility (4). The three elements of muscular fitness are strength, muscular endurance, and power, and these are defined as "the ability of muscle to exert force," "the ability of muscle to continue to perform without fatigue," and "the ability or rate at which one can perform work," respectively (5). Dogs need muscular fitness to walk and trot, sprint, slow down and stop, jump up and down, navigate obstacles and uneven surfaces, and stand on their hindlimbs. While the evidence for the role of muscular fitness in dogs is predominantly limited to rehabilitation from injury, disease, or age-related decline (6–8), there is extensive human research on the benefits of muscular fitness for health, injury risk reduction, competitive performance, and task performance (9–11).

Attaining the necessary level of muscular fitness involves determining the current level through assessment, pursuing an appropriate training program, reassessing periodically, and repeating these elements until the required level is reached. For optimal utility, an assessment should be reliable (measurement and test–retest), use a minimum of equipment, and be simple to perform (12). Muscular fitness in dogs is primarily assessed through daily task performance and muscle mass measurement (13–15), and other performance-based assessments have been proposed (16–18). Several canine sporting events (e.g., Fast CAT and Dock Diving) are highly standardized performance assessments that require significant contributions from muscular fitness. Muscular fitness is assessed in numerous ways in humans, and some competitive events are direct assessments of these modalities (e.g., track and field). Performance on short-distance sprinting, for example, is highly correlated with measures of power (19), strength (20), muscular endurance (21), and with trunk muscle mass (22). Off the athletic field, muscular fitness is also critical for human tactical athletes, those personnel in law enforcement, the military, or rescue services (23–26). Assessment of muscular fitness for these athletes is common, with the Army Combat Fitness Test including assessments of strength (3 Repetition Maximum Deadlift), power (Standing Power Throw), and muscular endurance (Hand-Release Push-Up, Sprint-Drag-Carry, and Plank) (27).

A gap exists in determining the muscular fitness of dogs whose abilities exceed the capacity of current assessments. An objective, simple, muscular fitness assessment directed at high-performance dogs would aid in guiding dogs in the return-to-work or sport phases of rehabilitation and inform operational decisions like deployment and retirement (28). The Penn Vet Working Dog Center (PVWDC) Sprint Test (ST) was proposed as a method to fill this gap (4). The ST is as an assessment of a dog's ability to generate whole-body power during the acceleration phase (first 25 m) of sprinting. The propulsion for sprinting involves forelimb (shoulder flexion, elbow extension, and carpal flexion), trunk (spinal flexion, extension, and stabilization), and hindlimb (hip, stifle, and tarsal extension) components with the hindlimb supplying the majority (29–32). The ST uses video timing to estimate the duration to complete the distance, and this timing method has been shown to be as accurate as the fully automatic photo finish systems used in international human competition (33).

There were several purposes for this study. The first purpose was to describe in detail the ST setup and execution protocol that were introduced in our original methods paper (4). The next purpose was to develop a training program for novice evaluators and assess the measurement reliability for evaluators with a range of experience levels. We hypothesized that a suitable training program could be developed to quickly enable a novice to measure ST performance with nearly the same accuracy and precision as an experienced evaluator. The third purpose was to characterize the acceleration profile of a dog performing the ST and compare it to data from human athletes. We hypothesized that these would be similar. The final purpose was to evaluate the inter-day reliability and describe the performance of a small group of working dogs. We hypothesized that the ST would have a small amount of inter-day variation, and this population would show the expected biological variation in performance.

Materials and methods

Animals

All canine participants were either working dogs in training or working dogs performing detection research at the PVWDC (Philadelphia, PA, United States). Dogs conducted their normal training in addition to the study. On musculoskeletal examination by one of the study veterinarians, all dogs were considered sound.

To be included the dogs had to be one of the typical working breeds, over 0.67 years of age, free of any performance-limiting condition, and familiar with the ST process. Dogs were excluded from the study if they were under 0.67 years old, had a performance-limiting issue, were not present for the entire study, and/or were not familiar with the ST process.

ST setup and performance

The ST setup and performance procedure is described in detail in the Supplementary material and summarized in Figures 1, 2. Briefly, the ST is a 25 m sprint from which the dog starts in a stationary down position. A highly motivating reward is used to incentivize maximal performance. Three starting zones corresponding with three start and finish lines are used to accommodate the dog's behavior (weight shifting or small movements forward) when highly aroused. The start and finish are captured on video [at least 60 frames per second (fps)] using recording devices synchronized by viewing the light from a flashlight. The dog performs 3–5 attempts, and the performance on the fastest attempt is designated as the test result. The dog's perceived effort [graded on a four-tier declining scale from highly motivated, "A," through decreasing visible effort ("B" and "C") to failed to complete, "F"] and test quality [graded on a four-tier declining scale from no issues "A," through increasing issues ("B" and "C"), to unable to complete "F"] are observed and reported.

ST measurement

The ST measurement procedure is described in detail in the Supplementary material and summarized in Figure 2. Briefly, the

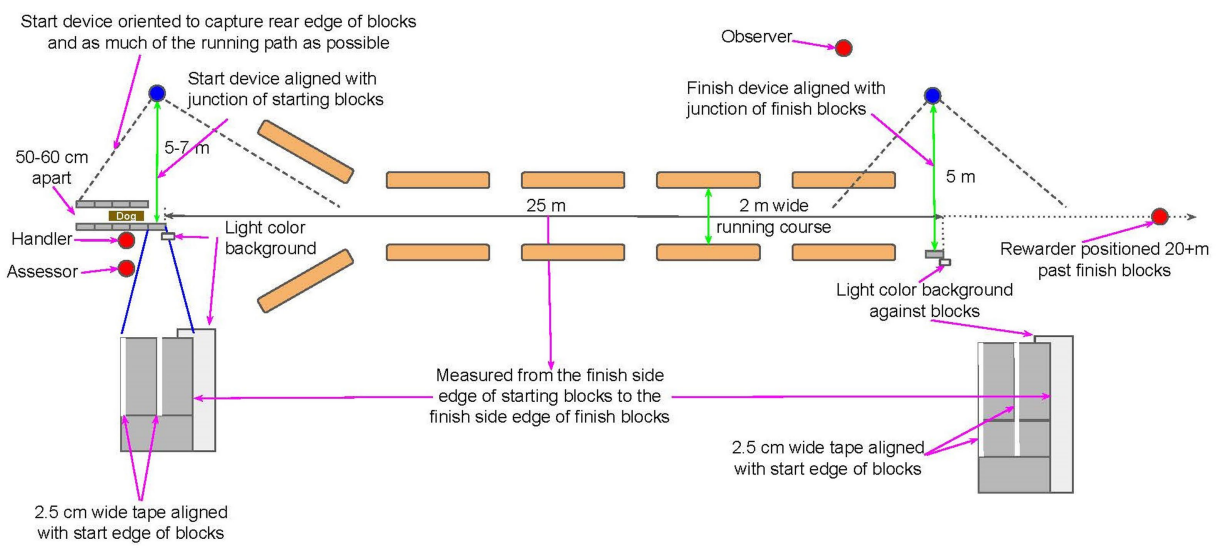

FIGURE 1
Overview of the ST setup.

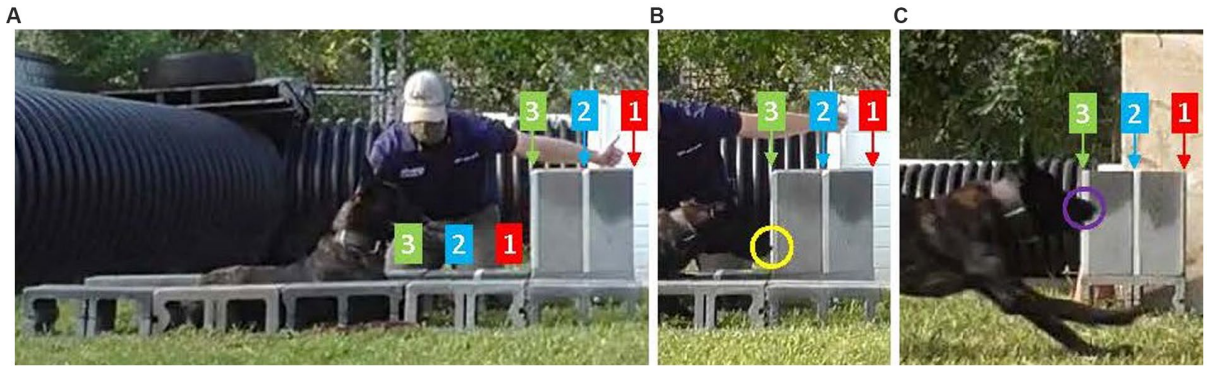

FIGURE 2
(A) Shows the ST start setup with the dog's chest in Zone 3. **(B)** Shows the dog's nose crossing start line 3 (yellow circle). **(C)** Shows the finish setup with the dog's nose crossing finish line 3 (purple circle).

start and finish videos are analyzed to determine the dog's quantitative and qualitative performance. This study used Kinovea (version 0.8.27), a free 2D motion analysis software running on desktop computers to perform frame-by-frame analysis. Both videos were analyzed to determine the frame when the synchronizing method was first visible. The start and finish videos were analyzed to determine the start and finish times of each attempt. Any abnormalities in the dog's movement or unrecognized perceived effort or test quality issues were noted. The duration of each attempt was calculated by adjusting the start time (Ts) and finish (Tf) times by the synchronize time (STs and STf) and then subtracting the adjusted start time from the adjusted finish time [e.g., (Tf-STf) – (Ts-STs)]. The shortest duration from a properly performed attempt ("A" for both perceived effort and test quality) was then reported as that dog's result.

Measurement reliability

Four raters were recruited for measurement reliability. One rater was to be highly experienced with measuring the ST (had rated more than 200 dogs performing the ST), one rater was to be moderately experienced (had rated 25–100 dogs performing the ST), and two raters were to have no prior experience measuring the ST before the training procedure.

Training procedure

The highly and moderately experienced raters measured the start and finish videos of five previously recorded dogs performing three attempts each. The highly and moderately experienced raters' measurements for the STs, STf, and the Ts and Tf from each attempt for each dog were compared. The two raters discussed any differences

and agreed on the actual measurement that would serve as the standard for the novice raters.

The novice raters were provided a standardized measurement protocol and a demonstration video to familiarize themselves with Kinovea. The novice raters reviewed the protocol and demonstration video and then discussed any questions with the highly experienced rater. After becoming familiarized with the process, the novice raters measured a training set of videos. They were provided with the start and finish videos of the same five dogs as above, and for efficiency of review, a spreadsheet identifying the approximate start and finish times of each attempt (rounded down to the nearest second) and the starting zone for each of the three attempts for each dog. Both novice raters measured each video to identify the STs, STf, Ts, and Tf for each attempt. These times were recorded to the millisecond, entered into an online form, and submitted. Both novice raters then measured the five dogs' videos again in a different order after an interval of 3 days.

The initial and repeat results of each novice rater were then compared to each other and to the measurement standard set by the highly and moderately experienced raters. The novice raters were provided feedback and provided the opportunity to remeasure any incorrect measurements. All raters were considered trained when 80% of their measurements exactly matched the measurement standard, and any measurements that did not match differed by only one frame in either direction.

Measurement reliability procedure

All 4 raters were provided with the start and finish videos of 23 dogs performing a total of 83 attempts (3–5 per dog) and a spreadsheet with the information for each attempt like that provided during the training procedure. Each rater measured all the attempts in each video and submitted their results using an online form. Three of the raters then measured all the attempts in each video again in a different order after an interval of 7 days.

The inter-rater and intra-rater differences for all measurements (STs, STf, Ts and Tf for each attempt, and the calculated duration for each attempt) were assessed. Due to the small variation in each of these individual measurements, the calculated duration for each attempt was selected for further analysis. This analysis was conducted in R (version 4.1.2) (34), and intraclass correlation coefficients (ICC) were determined using the IRR package (version 0.84.1) (35) based on a single observation, consistency, one-way model. The inter-rater reliability was determined by calculating the ICC for the first measurement attempt for all raters. The intra-rater reliability was determined by calculating the ICC for the initial and repeat measurement for each rater. The actual inter-rater difference was determined by first averaging the duration for each rater's first measurement to create a reference standard. Next, the absolute difference between this value and the duration for each rater's first measurement was determined. Finally, this difference for all raters was averaged for each attempt, and the overall mean and standard deviation were determined.

Acceleration profile characterization

Two dogs were recruited for this portion of the study. All ST attempts were performed in one session in the same location, and the dogs were positioned to start from the first starting zone. Additional identical video cameras and narrow, vertical, and high contrast marking lines were placed at 5 m intervals along the course at 5, 10, 15, and 20 m. All video cameras were synchronized, and each dog performed attempts until three attempts with both "A" perceived effort and test quality scores were obtained. The resulting attempts were measured using just the start and finish cameras by a single rater (BF) to determine the attempt with the shortest duration. All videos obtained for this attempt were then reviewed and the dog's progress was measured to determine the time to reach each marker. The duration between markers, average velocity (v) for each 5 m split, maximum velocity (v_{max}), and percent of maximum velocity ($\%v_{max}$) were then calculated.

Inter-day reliability

Eight dogs were recruited for this portion of the study. Two ST iterations were performed 1 week apart on the morning of the first training day of the week prior to any other training. No attempt was made to standardize or restrict the activities on the days preceding this first training day. Both iterations took place at the same location and involved the same personnel filling the same roles for each dog. Each dog performed attempts until three attempts with both "A" perceived effort and test quality scores were obtained. The resulting videos were measured by a single experienced rater to determine the attempt with the shortest duration from each iteration. To ensure measurement reliability, the videos of two of the eight dogs from each iteration were randomly selected for measurement by another rater. The results of the two raters were analyzed and compared to the inter-rater reliability data obtained during the measurement reliability procedure. The shortest durations from both iterations for each dog were compared, and the relative (ICC) and absolute (coefficient of variation (CV)) reliability were calculated. The ICC analysis was run in R using the IRR package based on a single observation, agreement, two-way mixed effects model.

Results

Refinement

The revised setup and execution protocol leveraged nearly 2 years of utilization, hundreds of canine athlete testing sessions, and thousands of attempts to bring needed clarity to the ST. The original description of the ST utilized a single camera at the finish to identify the first motion of the dog. This method was determined to be inaccurate due to the challenges with identifying small motions from over 25 m away. Early unpublished research to develop measurement training using this method showed inter-rater and intra-rater actual differences of approximately 0.1 s each. Refinement was needed to measure the ST more precisely and accurately.

The first refinement was adding a second camera at the start to better visualize the dog's initial movement. A simple method to quickly synchronize the two cameras was needed, and the shared light flash was introduced. This second camera allowed greater visibility of the dog's movements before and during the start, and a method to accommodate these movements and more precisely measure the start

TABLE 1 Average split velocity (v), split velocity relative to maximal velocity (%v_{max}), overall time, and overall average velocity (v) for each dog's fastest attempt.

	0–5m		5–10m		10–15m		15–20m		20–25m		0–25m	
	v (m/s)	%v_{max}	v (m/s)	%v_{max}	v (m/s)	%v_{max}	v (m/s)	%v_{max}	v (m/s)	%v_{max}	Time (s)	v (m/s)
Dog A	5.21	54.7%	8.33	87.5%	9.09	95.5%	9.52	100.0%	9.52	100.0%	3.16	7.91
Dog B	4.81	48.6%	8.06	81.5%	9.09	91.8%	9.90	100.0%	9.90	100.0%	3.22	7.76

had to be developed. In the original version, dogs did not always start oriented parallel to the running path. The concrete block channel structured the dog's position, and the starting zone concept associated with these blocks accommodated the inevitable movement of motivated dogs better than a single line. When viewed at 60 fps, dogs showed several starting movement patterns. Some moved their forefoot first, some dropped their head first, and some moved their entire body as a unit in a forward and upward motion. The starting point was therefore shifted from the "first motion," and the nose was selected to be a readily identified marker crossing a newly developed starting line. This line had to be close enough to the dog to still capture much of the initial movement off the ground while also being far enough away to not be already crossed by a dolichocephalic dog positioned at the front of the zone and leaning forward. The size of the concrete blocks assisted in this process, and the starting line is generally 20–40 cm away from the dog's nose when they first begin moving. This distance is covered in 5–10 frames and enables clear identification of the starting point while minimizing the lost portion of the dog's initial movement.

The ST was originally developed for young working dogs in training, and their behavior during early testing iterations mandated several further refinements of the protocol. Many dogs were challenged to perform a down position in a specific 20 cm zone while anticipating a sprint toward their favorite reward. Two additional zones and corresponding starting and finish lines were added, giving dogs more flexibility to perform the task successfully. The visual and physical barriers were added along the running path to reduce distraction and encourage straight movement. Dogs showed varying levels of motivation during the ST, and human error was also found to affect the dogs' performance. The perceived effort and test quality metrics were developed so that this qualitative information could be observed, scored, and recorded to add context to dogs' quantitative performance.

Measurement reliability

The highly experienced evaluator was a veterinary researcher who developed the measurement method in this project and had previously measured approximately 250 dogs performing approximately 1,000 attempts. The moderately experienced evaluator was an undergraduate research intern who had previously measured approximately 50 dogs performing approximately 200 attempts. This evaluator did not measure the videos a second time, and her results were therefore only included in the inter-rater evaluation. The two novice evaluators had observed the ST being performed but had no experience measuring the videos prior to the training procedure.

The novice raters reached the training standard after one round of remeasuring the videos (approximately 3–5 total attempts) they measured incorrectly during the first round. The inter-rater ICC for all raters was 0.995 (excellent) with a measurement duration difference of 0.014 (+/− 0.008) seconds (or 1–2 frames). The intra-rater ICC for the highly experienced rater was 0.997 (excellent) with a measurement duration difference of 0.015 (+/− 0.017) seconds. The intra-rater ICCs for the two novice raters were 0.997 and 0.996 (both excellent) with measurement duration differences of 0.018 (+/− 0.015) seconds and 0.022 (+/− 0.019) seconds, respectively.

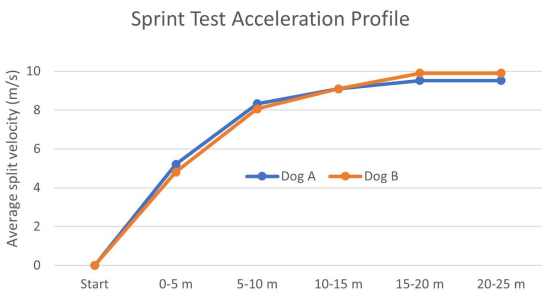

FIGURE 3
Acceleration profile of the average split velocity for each dog's fastest attempt during 5m segments of a 25m sprint.

Acceleration profile characterization

Two Labrador Retrievers participated in this portion of the study. Dog A was a 0.8-year-old intact male that weighed 27.9 kg, had a body condition score (BCS) of 3/9, was 61 cm tall at the withers, and was in training to be a search and rescue working dog. Dog B was a 3.0-year-old spayed female that weighed 21.6 kg, had a BCS of 6/9, was 55 cm tall at the withers, and was performing detection research. Both dogs achieved their v_{max} during the 15–20 m split and maintained that velocity during the 20–25 m split. The acceleration profile for both dogs is summarized in Table 1 and Figure 3.

Inter-day reliability

Three German Shepherd Dogs, three Labrador Retrievers, one Dutch Shepherd, and one Small Münsterländer participated in this portion of the study. Five dogs were in training for working careers (dual purpose patrol and detection law enforcement, single purpose detection law enforcement, or urban search and rescue), and three dogs were performing detection research. All dogs were intact, five were male, and three were female. The median age was 2.2 years (range 0.8–5.7), the weight was 29.0 ± 5.0 kg, the BCS was 4.3 ± 0.9 on a

9-point scale, and the height at the withers was 62.3 ± 4.8 cm. The shortest duration of each dog's best attempt across both iterations was 3.06 ± 0.098 s. The inter-day reliability ICC was 0.62 (moderate) with a CV of 3.2%.

Discussion

All dogs should be fit enough to perform their life, sport, and/or work tasks, and muscular fitness is an important aspect of fitness. Sporting and working dogs require a higher level of muscular fitness, and the PVWDC ST has been proposed as a method to assess this fitness in a simple manner suitable for the temperaments common to these dogs (4). In this study, we described the protocol to set up, perform, and measure the refined ST. We demonstrated the excellent intra-rater and inter-rater measurement reliability possible using this method, explored the acceleration profile of the ST, and captured the performance and inter-day reliability of a small group of working dogs.

The measurement reliability portion of this study showed the refined ST could be measured with excellent measurement reliability and actual inter-rater and intra-rater differences of 0.02 s or 1–2 frames at 60 fps. These results were obtained by naive raters after undergoing a short, standardized training protocol. Like other video measurement studies, the ST can be measured to the limit of the frame rate of the recording device (33). This precision is critical when identifying small changes in performance and muscular fitness.

We developed the ST as a measure of muscular fitness and aimed to capture most of the initial acceleration without the influence of top speed or speed endurance. A group of average human sprinters achieved 96.2% of their maximum velocity in the 10–20 m split and 97.1% in the 20–30 m split (36). The current male 100 m world record holder, Usain Bolt, achieved 80.4% of his maximum velocity in the 10–20 m split and 90.2% in the 20–30 m split of his 2008 Beijing Olympic Games performance (37). When developing the ST, we selected the 20–30 m distance and created the 25 m distance to balance the capture of acceleration performance with a distance likely to fit (with additional space required before and after this distance) within the limitations of existing fenced training areas. Various distances have been used in human athletics to evaluate sprint performance and correlate that performance with other fitness and performance metrics (38–41). The current ST is a first step toward that goal for canine athletes.

To our knowledge, the acceleration profile of sprinting dogs has not been previously characterized. The acceleration profile of the two dogs in this portion of the study was more like that of average adult human sprinters than the current world record holder (36, 37). These dogs reached their maximum velocity in the 15–20 m split and then maintained this velocity through the 20–25 m split.

A small group of mature working dogs provided initial information on the ST. The inter-day reliability for this population in this study was 0.62 (ICC) and 3.2% (CV). To our knowledge, the inter-day reliability of canine sprint performance has not been reported. The accepted standard for similar distances in human athletics is ICC > 0.75 and CV < 3.0% (39). The causes of the lower inter-day reliability of the dogs in this study are not known. We hypothesize that variations in physical activity in the days preceding the testing days (over the weekend) and subtle alterations in test personnel behavior (especially the rewarder) may have contributed. The performance of the dogs in this study was 3.06 ± 0.098 s, and this information can assist future research and inform the development of performance standards.

The primary strength of this project is the description of a method to assess canine muscular fitness in the field environment. To our knowledge, the ST is the first standardized measure of sprint performance and muscular fitness in canine athletes. The measurement reliability portion showed that naive raters could be trained to effectively measure the ST. Finally, the inter-day portion of this project provided pilot data on performance and inter-day reliability to inform future research. This project can serve as the launchpad for future measurement of sprint performance and correlation with sporting and working ability (42).

The limitations of this project involved the number and type of dogs involved in the inter-day reliability portion. The dogs participating in this portion of the study were a convenience sample of lean medium and large breed working dogs in training and dogs performing detection research. Thus, their best performance and inter-day reliability may not be reflective of other canine athlete populations. The ST methodology should be evaluated in dogs of varying sizes and temperaments. We did not compare ST performance with other measures of muscular fitness. Future studies could address these limitations by conducting the ST on large groups of working dogs performing similar tasks (e.g., military working dogs or dual purpose law enforcement dogs) and by comparing ST performance with other measures of muscular fitness, including thigh circumference, thigh circumference relative to limb length, and measurements of muscularity using imaging techniques.

The ST appears to be an effective way to assess the muscular fitness of a canine athlete in a field setting. The ST could be used before and after a muscular fitness training program to determine the effect of the program or to compare the effects of different programs. Performing the ST on a large group of similar dogs could illuminate potential effects of age and career or sport duration on performance and assist in establishing population-specific standards. The ST could be used serially during the return-to-sport or work phase of rehabilitation to stage recovery and inform utilization, competition, or retirement decisions.

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 Institutional Review Board of the University of Pennsylvania Institutional Animal Care and Use Committee.

Author contributions

BF, MR, and CO contributed to the conception and design of the study. BF, JG, RT, and SZ collected the data. BF and AM performed the statistical analysis. BF wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2023.1217201/full#supplementary-material

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The health of working dogs in conservation in Africa

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Introduction: Dogs are increasingly being employed for conservation purposes worldwide. In Africa, they work in challenging environments with unique health risks which have not been investigated until now.

Methods: To understand the health challenges faced by the dogs, semi-structured interviews were conducted with participants from 14 organisations that used working dogs in their conservation programmes. The data was qualitatively analysed by thematic analysis.

Results: Five themes were generated. Three affective themes influenced how participants responded to the challenges associated with having a successful conservation dog programme. A strong handler-dog attachment, proficient handler training, and the acknowledgement of the challenging environment were pivotal to maintaining dog health. Two themes related to the difficulties in managing these programmes and how veterinary support interacts with the management choices being made.

Discussion: To have healthy conservation dogs, current and future programmes should focus on fostering the handler-dog relationship and provide continuous handler training. The management of conservation dogs' health should adopt an evidence-based approach. Future research should focus on areas where the evidence base is lacking, particularly in the areas of prevention and treatment of African canine trypanosomiasis. Programmes should develop a good working relationship with a veterinarian that has access to evidence-based veterinary medical information.

KEYWORDS

canine, conservation, Africa, health, thematic analysis, African trypanosomiasis, heatstroke

1. Introduction

The remarkable olfactory capabilities of dogs (*Canis familiaris*) combined with the ability for humans to develop close working relationships with them means that they are employed for a wide variety of tasks. Dogs are employed by conservation programs and governments worldwide for wildlife population research, monitoring, management and in preventing and enforcing wildlife crime. They are used in the detection of elusive wildlife and their scat, in invasive species detection, carcass detection and the detection of disease (1–6). In many African countries, handler-dog partnerships are used to support conservation efforts in the detection of elusive species and to track poachers from a crime scene and in detecting illegal firearms, snares and illicit wildlife trade such as ivory, pangolin scales, rhino horns and bushmeat at local roadblocks and international ports and borders (7, 8). Livestock guarding dogs are also employed by conservation organizations involved in the mitigation of human wildlife conflict (9). They

play an important role in conservation, but their management is distinctly different to that of other conservation dogs which is beyond the scope of this study.

These roles require the development of complex skills on the part of both the handlers and the conservation dogs. Handlers must be skilled in reading the body language of their dogs and comfortable in the environment in which they work (10). Dogs are more accurate at detection when working with familiar handlers than with unfamiliar handlers (3, 11, 12). The dog-handler relationship is therefore an important component of the success of the program. The training of both dogs and handlers and development of the dog-handler relationship requires extensive work for the program to be successful (13).

Conservation dog partnerships are relatively under researched, with limited information regarding how dogs are sourced, trained, and partnered. The cost of investing and maintaining a program with dogs working in conservation is also unknown. Experienced trainers are required to select prospective dogs that are in good health with suitable traits and train them in their deployed location for their required task (14). In Africa, dogs are often deployed to remote locations from abroad and trained by foreign trainers, adding further to the cost of the program. Published guidelines on conservation dog selection, training and management mainly focus on the behavior and training of dogs. Very little has been published on the management of their health, other than recommending that dogs selected are healthy and of a suitable build for the environment in which they work (14, 15). However, the importance of optimizing conservation dog health cannot be underestimated. If a dog cannot carry out the work for which it is trained, then not only does it impact the welfare of the dog, but it is also the loss of an asset and loss of resource investment. In fact, canine health status is known to directly affect the olfactory capabilities of dogs upon which these programs largely rely (16).

There are very few published guidelines on the health and welfare needs of conservation dogs. A review of conservation dog programs in Africa in 2015 by the non-governmental organization Working Dogs for Conservation made recommendations for best practice for conservation dog programs, highlighting the main challenges faced from conservation dog programs, the infrastructure and the expertise that is required to establish and maintain a successful program. Recommendations that directly linked to conservation dog health included the construction of appropriate kennels and training handlers in veterinary first aid. Inadequate veterinary care was identified as one of the main challenges that conservation dog programs in Africa faced (17). The New Zealand Department of Conservation Dog/Handler Team Standard Operating Procedures gives brief guidelines on the minimum care of conservation dogs, advising adherence to the national legislation Animal Welfare (Dogs) Code 2010, vaccination and regular parasiticide treatment (18). This ensures that the health of conservation dogs is considered by those working in the industry but does not address the concerns that conservation dogs may face due to their unique circumstances.

The veterinary intervention and care required to support these partnerships is necessarily based on other working dog partnerships that are more clearly understood. Military working dogs and search and rescue dogs also perform detection and apprehension work and may have to do this in harsh terrain, facing temperature extremes and geographical isolation so are comparable to conservation dogs (19, 20). However, one must be aware of key differences between conservation dogs in Africa and other working dogs. There are possible differences in breed composition of conservation dogs compared to military working dogs. Military working dogs and search and rescue dogs often are well funded whereas conservation dog programs are often financially constrained (13). Military working dogs are deployed to challenging environments for shorter periods, whereas conservation dogs are more likely to be permanently deployed in a challenging environment for their entire working lives.

American military working dogs undergo health screening before acquisition including physical examination, hematology and serum biochemistry tests, infectious disease screening and hip and elbow screening for joint dysplasia. Female dogs are neutered at admission. Throughout their lives they undergo biannual physical examination, hematology, biochemistry and urinalysis and have regular vaccinations and parasite control. Handlers undergo basic veterinary first aid training with the opportunity for further training throughout their career (21). Similar recommendations were made by Jones et al. for search and rescue dogs (22). It was also recommended that individual programs formulate individualized veterinary health plans with the veterinarians to whom the dogs are registered and that handlers be trained in emergency first aid (22).

Examining the problems leading to loss of workdays, retirement, death and euthanasia in the military working dog and search and rescue dog literature can inform on health challenges that conservation dogs may face. Conservation dogs in Africa are likely to face similar challenges in addition to challenges unique to their specific location on the continent. A study on dogs deployed to Iraq in a 20-month period examined the reasons for 1,530 non-combat related veterinary visits in military working dogs. Dogs had dermatological problems (25%), soft tissue trauma (21%), gastrointestinal disorders (17%) and musculoskeletal disorders (14%) (21). A small survey on search and rescue dogs deployed to Haiti in response to the 12th January 2010 earthquake also revealed a range of minor conditions that the dogs succumbed to, with soft tissue trauma and dehydration the most commonly identified (23). Additionally, conservation dog programs in Africa may also be challenged with tick-borne disease, canine African trypanosomiasis, snake envenomation and poisoning secondary to accidental ingestion of poisoned wildlife (24–27).

Africa was targeted for this study due to the scarcity of research into the health and welfare of conservation dogs in this region. This is of particular concern due to the valuable work that the dogs conduct combined with the challenging environments in which they are placed. In this study, we sought to characterize the health challenges faced by programs using conservation dogs in some African countries, and how they are managed, particularly around the provision of veterinary care. The overall aim in this study was to explore the difficult challenges and develop key considerations and recommendations for those deploying dogs within African countries and highlights the areas required for future research that would most benefit the welfare of conservation dogs in African programs and elsewhere.

2. Materials and methods

2.1. Position of researchers

A qualitative research approach was adopted to explore health challenges and management approaches of African conservation

programs that use conservation dogs. This approach allowed the understanding of the key health challenges that conservation dogs faced and the complexities around managing such programs (28). The semi-structured qualitative research interview was used to gain rich and detailed information about the health challenges that conservation dogs face, and the methods of management adopted by the programs. This enabled health challenges and management decisions that are complex and influenced by multiple factors to be understood. It also identified the social factors that affected conservation dog health (28, 29).

Thematic analysis was used to identify patterns in the data and thus extract main themes. Themes identified from the data allowed for the perspectives of the participants to be communicated directly from their own experiences, rather than using predefined parameters (30).

The three principal leads were NEE, a white female cisgendered practicing veterinarian with experience of the working dog literature. NA is a white male cisgendered veterinarian with experience working in southern and eastern Africa in applied conservation research. JM is a white female cisgendered non veterinary author with experience working in veterinary associated fields. She identifies mainly with a socio-constructionist epistemological position.

The methods were reported in line with the consolidated criteria for reporting qualitative study guidelines developed by Tong et al. (31) to provide a transparent comprehensive report. Per Braun and Clarke and Tong et al. quotations were used to support the research position (32).

2.2. Data collection

Purposive and snowball sampling was used to identify and select participants. Participants were selected if they managed a fully operational conservation dog program in Africa or were training dogs for deployment to conservation dog programs in Africa. An online search engine and professional contacts were used to find the programs. Participants were contacted through their email address or contact page with the same email template (See Supplementary material). Participants also informed the interviewer of other programs that were not originally found through an online search, and these programs were contacted through their contact page on their website, email address published by their website or through informal introduction by the participant. Prior to the interview, participants received an email outlining the aims of the interview, the consent and withdrawal process (See Supplementary material). Interviews were conducted face to face or through an online call between 12th November 2019 to 30th March 2020. Fifteen interviews were conducted from fourteen different conservation dog programs. There were ten heads of program, three trainers, two handlers and two veterinary surgeons. More than one participant was interviewed per organization if it was deemed appropriate by the participant and interviewer for acquiring the most detailed information.

Semi-structured interviews using an interview guide were conducted. The interview guide was piloted before use with colleagues with experience in the field. The topics included in the interviews covered the structure and purpose of the organization, day to day work and care of the dogs, routine healthcare, health problems encountered including mortalities, infectious disease and non-infectious disease, the availability of veterinary and laboratory skills and facilities, and facilities or competences desired by the program to improve their conservation dog's health (Table 1). The interview was conducted by a 32-year-old female white British veterinary surgeon (NEE), with no other people present at the interviews. The interviewer had had previous non-professional contact with two of the participants (CD8 and CD9b). The interviews lasted an average of 69 min (±21.5 min) and ended when all the available information was gathered. The interviewers were also invited to share photographs and further information following the interview. The iterative nature of the

TABLE 1 Main interview topics covered (see Supplementary material for the full Interview Guide).

Program structure	Number of dogs
	Breeds
	Nature of work
Husbandry	Housing and feeding
	Transport facilities
	Husbandry skills
	Working/training hours
	Vaccination protocols
	Parasite control protocols
	Dental care regime
	Health screening
	Record keeping
Health problems encountered	Most common health problem
	Most serious health problem
	Infectious disease
	Trauma
	Heat exhaustion
	Poisoning
	Gastrointestinal disorders
	Behavioral disorders
	Chronic conditions
	Unidentified disease
	Main causes of mortality/retirement
Conflict encountered	Dog-wildlife conflict
	Dog-human conflict
Veterinary facilities	Veterinary facilities available
	Laboratory facilities
	Accessibility to veterinary and laboratory facilities
	Veterinary capabilities of staff
Areas that would benefit from further training or investment in facilities	Husbandry facilities
	Husbandry skills
	Veterinary facilities
	Veterinary skills
Anything that is particularly important	

qualitative research interview meant that the focus of some questions altered as more was learnt about the subject. For example, the factors affecting the dog-handler relationship were discussed in much more depth as its importance was brought into focus.

The interviews were recorded with Otter.ai software (AI Sense Inc. 2019) transcribed verbatim by the interviewer with additional information attached to each transcription document. The transcript documents were sent back to the participant for comment, nine participants responded to the invitation to comment on transcripts with few or no comments and five did not respond. The results were disseminated to the participants in the form of short, easy to read summaries.

2.3. Data analysis

The transcriptions were coded by the interviewer using NVivo12 software and themes were generated from the codes and then refined (32, 33). The themes were sent to the co-authors for sense checking before and after refinement. NEE was primary analyzer- from the position of an informed perspective as a veterinarian with an understanding of the literature. Per Braun and Clarke a theoretical approach was taken to the taken with engagement with the literature prior to analysis. In this way authors brough *a priori* ideas to their interpretation of the themes (32).

2.4. Ethics statement

The project was approved by The University of Edinburgh Human Ethical Review Committee (Reference HERC_410_19). To gain approval, all efforts were made to minimize harm to participants sharing potentially sensitive information about their activities with the working dogs and the welfare challenges that were in place. Participants were anonymized and individually identifiable information was withheld. Informed consent was required before recording the interviews. Participants were informed that although every effort was made to make them identifiable was made, they may still be identified due to the small number of conservation dog programs. Participants could withdraw from the study at any time and the contact details of the first author were made readily available.

3. Results

3.1. Overview of participants

The overall structure of the programs that participants represented is summarized in Table 2. Thirty-two potential participants were contacted. Fourteen organizations in total were interviewed (44%), with remaining organizations either not responding (31%), initially responding but without further contact (19%) or not having a conservation dog program running at that time (6%). All organizations interviewed were in southern and eastern Africa. Fourteen interviews involved only one participant, and only one interview per organization was conducted apart from CD10 whereby the handler and veterinary surgeon were interviewed separately for logistical reasons.

TABLE 2 Summary of participants.

Program attribute	Summary
Length of program	Range 3–31 years
	Median 7 years
Number of dogs	Range 30–40 dogs for training programs
	Mean 4 dogs for conservation organizations
Breeds	Range Belgian Malinois, bloodhound, Labrador retriever, pointers, Hanoverian hound, German shepherd, spaniel, Weimaraner, medium crossbreeds.
	Mode Belgian Malinois
Age of acquisition	64% acquire adults, 22% puppies, 14% a combination.
Health check	57% preadmission health checks, 36% no health check, 7% ensured health of parents
Type of work	44% detection and tracking,
	14% species detection,
	14% tracking only,
	14% detection, tracking and apprehension,
	7% tracking and apprehension
	7% detection only

3.2. Thematic analysis

Five major themes were generated from the coded data, three affective themes which influence how participants responded to the challenges associated with this kind of work and characterized their motivations and feelings in the role. The final two themes related to the difficulties in managing these programs and how veterinary support interacts with the management choices being made. The themes are:

1) Strength of handler-dog relationship
2) Importance of handler training
3) Acknowledgement of risk to dog
4) Challenges in management
5) The relationship with veterinarians

In summary, the challenges in each aspect of the conservation program demonstrate how difficult it can be to have a functioning conservation dog program, resulting in dogs that accumulate high value. From acquisition to training of both handlers and dogs, through to management and then finally retirement, participants spoke of similar challenges along the way (Table 3). Upon overcoming these challenges and then seeding the dogs succeed,

TABLE 3 The challenges of launching and maintaining a conservation dog program.

Aspect of conservation dog program	Challenges
Acquisition	Expense, logistics, loss of asset and waste of resources if dogs are discharged or early or die.
Handler selection and training	Handlers have no prior experience, skill required in selecting handlers.
Dog training	Logistics and cost of initial intense training, maintenance training and corrective training.
Health management	Limited evidence base, difficulty acquiring medications, cost of medications, cost of evacuation, fear about side effects of prophylactic regimes, difficulty finding competent veterinary care, limited access to specialist care, infectious disease, heat stress.
Program management	Skill fade, maintaining handler performance and morale, dog security, deployment to remote sites, evacuation.
Retirement	Logistics of retirement, availability of retirement homes.
Accountability to donors	Pressure to be productive.

handlers formed strong relationships with their conservation dogs, and were highly motivated to support each dog as an individual. This was strengthened by a wide recognition of the dangers to the dog inherent within the work, such as the effect of temperature extremes. Participants viewed handler selection and training as key to this relationship, particularly in supporting dogs in these high-risk roles. Due to the high investment level in the handler and the strength of relationship between the dogs and handlers, a major concern was how to appropriately prevent, diagnose and treat disease in order to obtain the maximum benefit from the handler-dog relationship. Program staff relied on management practices that did not always have a strong evidence base in veterinary medicine. Program staff found it difficult to find and evaluate evidence related to the dogs' management. In areas with trypanosomiasis, it was a major concern as it there is no way to eliminate the risk of infection, diagnosis was not always feasible in remote locations with no veterinary diagnostic capabilities, treatment was often unsuccessful and if the dog did survive, infection often resulted in long term effects resulting in the requirement for the dog to retire from work. Compounding this was the challenge of establishing a trusting relationship with veterinarians. While a "good veterinarian" was perceived to be highly valuable because of the support that they gave to the valuable conservation dogs, participants that struggled to establish such relationships with veterinarians felt their dogs' health to be vulnerable and were frustrated by the inadequate care that resulted from the lack of access to a veterinarian that they trusted. Each of these themes reveals something of the challenges in managing conservation dogs in these settings and provides useful information regarding how the veterinary profession can support this unique working dog partnership.

3.2.1. Strength of handler-dog relationship

Trainers and heads of programs recognized the importance of fostering the handler-dog bond as handlers were heavily relied upon for managing the health of their dogs and minimizing the risks inherent in the conservation dogs' work. Overcoming the challenges of having a functioning conservation dog program as well as seeing the dogs succeed created a strong bond between the conservation dog staff and their dogs. This strong bond was demonstrated in one case by the suspension of the program after the accidental deaths of one dog and the lengths that another participant was prepared to go to in caring for his dogs despite budget constraints:

> *"But then during one of the training sessions the handler threw the ball quite a […] there was a giant hole and the dog fell headfirst into the hole and broke her back. And she ended up having to be euthanised. And we were borrowing her, we were not even renting her and it was devastating for us to have to contact CD7 [the trainer that the dog was on loan from] and he came up and said that she would not be happy so she ended up having to be euthanised. So then we took a year off, where we were trying to decide if this is something we really wanted to get into." CD11, Head of programme.*

3.2.2. Importance of handler training

All but three participants communicated that the handlers were central to the health of the conservation dogs although it should be noted that this was not explicitly asked within the interview schedule. Of the three participants that did not communicate this, two were handlers themselves and one was a veterinarian that was not involved in the day-to-day activities with the dogs. Participants described multiple factors resulting in the establishment and maintenance of a team of dedicated handlers selected from a cohort who had had no prior experience of handling dogs. They described the skill that it takes to select a team of handlers and train them to work with the dogs as well as foster a human-dog bond which results in them valuing their dogs as well as noticing subtle changes in their dogs' behavior that might be indicative of ill health.

> *"Because if the handler, I mean the handlers are like the basis of everything. If they are not operating the dogs, the dogs do not operate does not matter how good your dog is. And there's so many complex factors in there with the human from private stuff to work stuff to everything, you know." CD12 Head of programme.*

> *"I think key to the whole thing is having the right handlers. The handlers make or break them. And we are really fortunate we have got a really good team who really care about it. You have the right handler. A- you get a productive dog; B you know when it comes to welfare, you know they are on it early." CD13 Head of programme.*

Participants that had a selection and training process that resulted in dedicated handlers saw this as a major benefit for their dogs' health. The handlers were instructed that the health and welfare

of their dogs was their responsibility and of paramount importance. The health of the dogs should not be put at risk, even if the outcome of the work would be compromised. They were trained to recognize, prevent and manage heat stress, prevent dietary indiscretion, perform basic first aid and know their dog's individual character and thus recognize any health problems. They were trained to perform a health check or groom daily, which superficially served the purpose of removing ticks, checking for thorns and keeping the coat in good condition. On another level it gave handlers the opportunity to recognize early signs of ill-health. One a deeper level it fostered the dog-handler bond that was not necessarily culturally intrinsic in the handlers.

> *"And so yeah, so the I think that's one of the main things is the handler training is that, you know, the dogs, the dogs' health and welfare, and wellbeing is absolutely number one priority. And without that they do not have a job … there's just no question that the dogs do not get that temperature taken. There's no question that the dogs do not get groomed. There's no question you know, it's become muscle memory now for such a long period of time that it's not really questioned.." CD14, Head of programme.*

> *"Yeah like we groom every day. You obviously do not need to groom every day, but we do it every day. […]. Other dog guys come to me they are like, other dog people in general. They're like, that's a bit excessive, I'm like I absolutely agree with you. […] I have the sequence of grooming- again it's on my notice board. Everybody does the same thing […]and if you follow the sequence, you identify injuries on the dog, you can find parasites, you can find thorns and whatever. It's not about the grooming, it's about the process and maintaining a healthy working dog. So, but it's also rapport development." CD5 Head trainer.*

> *"So grooming every day. It's how we keep our dogs healthy to be honest. Spending time with the handler and they do the health check points. We have 10 health check points, and they groom and they have the connection and off they go." CD7 Head trainer.*

Additionally, participants recognized that without maintenance training there is a risk of skill fade which puts the whole program at risk, including the health of the dogs. Handlers invariably received initial training from experienced trainers, but the frequency of maintenance training whereby trainers returned to the program to deliver further training varied considerably. Infrequent maintenance training was seen to impact the performance of the dog-handler team negatively. CD14 recognized the value in employing a resident experienced trainer:

> *"I think other units have missed out on because they get that dip in and out. Somebody will install and then leave and then come back six months later and they fix all the behaviour and all the problems that have evolved during that six-month gap. Get everything right again after a couple of weeks and then they disappear for another six months and you keep getting that skill fade." CD14 Head of programme.*

CD12 recognized the value in regular visits from an experienced trainer:

> *"We have them at minimum, every two and a half to three months maximum four months, there is at least three or four visits a year, they are with us, where they spend two weeks with the handlers and the dogs. Just tightening up the screws and, and then adding every time adding to the learning to the training." CD12 Head of programme.*

3.2.3. Acknowledgement of risk to the dog

Participants described how they must balance the health risks that come with working and training against the requirement to train and work. High temperatures, high tsetse burden and rough terrain meant that dogs had to cease working or training at times, which could be seen in the short term as a loss of workdays, but without doing so could lead to disease resulting in a loss of many work days and even death. Snake envenomation, snake venom ophthalmia, minor injuries, intoxication and trauma were other threats to the dogs' health during work.

> *"It's really horrible, and then getting onto later that actually causes one of my main issues or not a main issue but it's just like, ya when you call like, it's like an acute health problem, so I get a lot of thorns in the pads, thorns in the bodies, I've had to pull the dogs because they cannot work in areas with thorns, it does not even help with booties because it was going into their body, and it's just not worth me having the dog out of work." CD3, Handler.*

All participants experience the challenges of working in extremes of temperature and it was taken very seriously. High temperatures dictated training times and even whether the dogs would be taken out at all.

> *"Sometimes we do not even take the dogs out. Depending on the temperature. The ground can get so hot you cannot put the dogs on the ground. Part of our training, all our field rangers are trained to feel the ground. It's not about just the air temp, it's about the ground temperature and it radiated temperature coming up from the ground." CD6, Trainer.*

Recognizing when dogs were showing early signs of heat stress and treating it was an important aspect of handler training. This adds to the importance of competent handler training in conservation dog health management and the responsibility placed upon handlers to safeguard their dogs' health.

> *"And I feel like our handlers are very, very in tune with heat. It was drilled into them, in the start of the program, just really intense training on, you know, just animal welfare for the first four months before they did anything else." CD4, Head of programme.*

Handlers were expected to recognize signs of early heat stress on an individual basis, as it was understood that individual dogs would express symptoms very differently and have varying levels of tolerance.

> *NE: "What about heatstroke? […]do you have problems with that?" CD2: "So currently not. I think it's because when I started with the guys it was a big thing on monitoring of dogs. I was really bad on that, if they did not do it I did not take it [well], and monitoring quite well and rehydrated dogs quite well so I have not had [heatstroke occur] yet. Close to it, yes, but the handlers quickly pick it up and do something about it." CD2, Head of programme.*

> *"When you see a bloodhound tracking and now, once he is not good, he leaves the track and tries to go to the bush. But also if you have the dogs you know, this dog will go certain kilometres you know that a certain dog can go 12 kilometres. But when they are on a real scenario, we give one 8 km, you remove and give another dog. We do not want the dogs to be more tired. Because you still need- when the other dog is tired you remove him again. So we make sure they get a lot of rest, a lot of water." CD10, Handler.*

Perhaps due to the focus on it during training, no participants reported deaths due to heatstroke. Only one participant described an episode of heatstroke in one of the dogs, discussing how it could be prevented in the future by reinforcing the common message from participants that each dog expressed heat stress in different ways with differing tolerance levels.

> *"You know, when this heat stroke happened, we definitely spent a lot of time discussing the symptoms of heat stroke if you are not paying enough attention properly, and it came up very quickly- it was only 10 min of walking away from the camp and not a strong exertion. And we know now it was the heart murmur was more than caused it than anything else so."…"We find when she is too hot she does not want to drink water, so even when we give her the right amount of breaks she tends to try to ignore you and pretend she's not hot, but we also tend to stop her when her temperature gets high now." CD11, Head of programme.*

These themes strongly impacted how participants considered challenges in their work, particularly around seeking out evidence for disease management, and the importance of their relationship with their veterinarian.

3.2.4. Challenges in management

Programs faced numerous challenges in the management of their dogs. This was compounded by the lack of evidence base in methods of mitigating these challenges. The absence of scientific literature on best practice, the difficulty obtaining correct medications and the reliance on other programs' experiences in informing their own practices led to programs having a weak evidence base behind their practices. The lack of evidence on best practice for the prevention and treatment of trypanosomiasis was the greatest concern for all programs in areas with a tsetse fly burden. Two programs actively avoided working in areas with a tsetse burden, five programs operated in areas with a tsetse burden and all three trainers had deployed dogs to areas with a high tsetse burden. The remaining five programs were not working in a tsetse fly area at all. The five participants that operated in areas with tsetse flies employed multiple preventative methods and expressed frustration at not knowing what methods were most effective.

> *"We are all just guessing? And if anybody can get anything, you know, for dogs in Africa with tryps [with a] bit more research, it would be great because then we would all feel- I just think his lack of confidence really anything else? We are just flying by the seat of our pants!" CD14, Head of programme.*

To address this evidence gap, programs employed multiple preventative methods in varying combinations including fly netting on vehicles and kennels, tsetse targets sprayed with insecticide, insecticide sprayed on vehicles, topical insecticide spray or lotions, regular isometamidium injections and screening for disease with daily temperature checks. One organization also performed regular blood smears to screen for disease. This high level of intensity of management required the dedication of trained handlers. No method was deemed most effective in their experience, but a combination of methods gave program managers the most confidence in preventing disease in their dogs.

> *"So, we are super fortunate, and the guys take it extremely seriously, the trypanosomiasis that tsetse issue. We are totally riddled with tsetses, our guys do not train and when they see tsetse flies around, they will move location until they get into an area where there aren't any tsetses, our dogs are treated all the time, they only move in fly proof kennels and boxes." CD12, Head of programme.*

All organizations ($n = 14$) using isometamidium long-term to prevent disease were concerned about long-term side effects, although it was not clear what these effects may in fact be:

> *"They get their prophylaxis for tryps and all that. I hate it, that there is no product out there for dogs. We mix Samorin with saline solution and half goes into each leg, to make it less viscous." CD5, Head trainer.*

> *"We started treating the dogs from the time that they were here with Samorin every 10 weeks. That is for tryps. And there's not a lot known about it as far as I know, because there's just not a lot of people doing that because there's not a lot of dogs in tryps areas, usually and if they; if they are there they go to treat them every 10 weeks and it is also because it's quite a hard medicine for them. So it's tough on the system." CD1, Head of programme.*

Another area where disease management faced challenges and lacked evidence was in its diagnosis. Participants described how they endeavored to find the cause of death and severe illness in their dogs and expressed frustration that this often was impossible. This often led to presumptive diagnoses. Delays in, or absence of veterinary attention due to isolation, poor sampling and inadequate processing techniques were to blame.

> *"And the circumstances are not 100% clear. The fact that he was poisoned that is, that is clear but how it happened yeah, did he pick up something by mistake was he wasn't on purpose we actually have never been able to establish we also could not find out what poison*

> *it was. Okay, even though all the contents of the stomach and blood and a lot of other samples are sent to different labs, but they could not decide." CD1, Head of programme.*

Programs expressed difficulty acquiring medications and some medications were too expensive for programs. This varied according to the country in which the program was based, but resulted in the same approach to solving the issue. Programs were forced to use less expensive and more easily acquired medication with a weaker evidence base behind their efficacy.

> *"For keeping parasites out of the kennels, we have a, I mean it's just spray. But at the end of the day when everyone enters our kennels we spray. It's a very thin mix of bleach because we cannot get F10 [in this country] it's a bit difficult, but it's a weak mixture of bleach spray, so everyone sprays when they get in" CD2, Head of programme.*

Participants admitted that they did not always pursue a diagnosis or have a strong evidence base behind their decisions when treating disease but would follow practices they felt would maximize the chances of recovery.

> *"We have not had a confirmed case of tick fever, but there have been times when a dog has been looking down and we have been giving them Berenil. So that will knock on the head tryps and/or tick fever. So there are cases where we have not had a blood test done. We just treat it straight away." CD13, Head of programme.*

> *"But generally the issue [with antivenom] is one the dose and you do not know how much and you are not sure what snake has bitten. So normally in the bush we are not sure, so what we do is this. We give a bucket of dexamethasone, I mean like 5x recommended dose. Our theory is that it slows down the metabolic rate, and anti-shock and anti-inflammatory and all these things. I do not know! The other thing is electric shock, we use a cattle prod. So if we can see where the bite is which can be quite hard, is we wet down the dog and put a muzzle on the poor fellow and we zap them. All I know is that when we do it they do not die." CD7, Head trainer.*

3.2.5. The relationship with veterinarians

Participants valued the relationship that they had with the veterinarians starting with the screening process at acquisition. Once deployed, they found it challenging to find veterinarians that they trusted to look after their dogs:

> *"getting proper vets- there are, but there's also some vets where you are like geez I do not know if I'm at the right place." CD2, Head of programme.*

The nine participants that had a good working relationship with their veterinarian(s) valued it highly. Apart from providing treatment, they communicated with their veterinarian(s) remotely, asking for advice and entrusted them to train their handlers in first aid. Programs that lacked a good relationship recognized this as a challenge to their dog's health as it prevents them seeking veterinary treatment and advice.

> *"I'm fortunate enough that if I call the vet now, I can get to my vets right away. I just need to keep my dogs alive up until then. And we have an open line with, with the vets we started a really good relationship with him where, if I phone him." CD2.*

Participants would go to great lengths obtaining veterinary care that their dogs required including emergency evacuation by aeroplane to veterinary facilities and even breaking the law:

> *"Anyway, so she got there, she was fine for months. But the vet here took out the ovaries but left a bit. And the uterus got infected and she got a pyometra. And the really cool [vets] are really great and switched on and South Africa is just across the border so they have lots of kit and it's all good. So they got her better from that, but she had a cyst on the back wall of her vagina, but it was too close to the back wall of her bladder, they were worried if they did anything they would f*** it up. So we needed to refer her to Jo'burg. So getting a dog into SA is a whole serious problem. So I called a dodgy mate and he smuggled her into SA and she reappeared in SA. Got her up there, my guy got her in Jo'burg. So they broke her pelvis to get at it and rewired it." CD7 Head trainer.*

4. Discussion

4.1. A qualitative study on the health of conservation dogs

This research study gave a detailed understanding of the health challenges faced by conservation dogs in four countries in eastern and southern Africa, and how they are currently managed. The semi-structured interview meant that aspects most important to the participants were focused on, which positivist research approaches would not convey (28). Qualitative analysis provides an integrative approach to understanding veterinary health problems, particularly those that are poorly understood (29). It allows the communication of scientific and non-scientific knowledge that participants rely upon to make decisions. The essentialist framework meant that the data communicated the reality of the situation. For example, preventing heatstroke is a constant battle- this is assumed to be true and constructs the reality of the situation. It may also allow researchers to learn from those immersed in this specialist field as well (34). Hall et al. acknowledged that those involved in training working dogs are not formally involved in research, and may not have scientific explanations for their practices, but have years of experience from which researchers and peers can learn and highlight areas requiring further applied research (35). This study is an example of the use of qualitative research in the acquisition of these experiences.

It must be understood however that this does not communicate the viewpoints of all conservation dog programs in Africa, and other programs may face different challenges according to their geography and program structure. Programs in western and northern African countries, non-English speaking programs and programs that were

not contactable by email were notably absent from the study and may have very different experiences. Programs that had historically been in place but had failed were also absent, so challenges contributing to the failure of programs could not be explored. Failing programs may also have been less likely to respond. However, the interview process allowed for participants to discuss their past challenges as well as current ones and describe how the challenges were overcome and did not lead to failure (36).

4.2. The handler-dog relationship

The challenges of having a functioning conservation dog program were multifaceted and ran from the initiation of the program, program management through to retirement of the conservation dogs. These challenges increased the inherent value of the dogs and thus placed a great deal of responsibility on the handlers to keep their conservation dog healthy. A strong handler-dog relationship was important in maintaining the health of the dogs demonstrated by the fact that all participants other than the handlers themselves and the veterinarian who was not involved in the day-to-day care of the dogs stated that it was fundamental to the success of the entire program. Handlers understood and knew dogs as individuals, noticing changes in their health at the early stages, thus allowing intervention before disease was severe. A strong relationship meant handlers prevented them from working in dangerous circumstances such as when it was too hot, or the tsetse challenge (and therefore the risk of contracting trypanosomiasis) was too high. Most studies on the relationship between working dogs and their handlers have found dogs to be in an improved welfare state, have less behavioral problems and for humans provide emotional and psychological benefits (37–41).

Handlers cannot thrive from a strong handler-dog relationship alone. For a strong handler-dog relationship, handler welfare must be prioritized alongside conservation dog welfare, taking a One Welfare approach (42). Handlers are responsible for the health and performance of valuable dogs in a challenging environment. This responsibility should be rewarded proportionately to this high expectation that is put upon them. A combination of logistical support and pastoral care is warranted for this group of specialized individuals that work in dangerous and challenging environments, while safeguarding the health of their dogs. Only recently has the welfare of rangers in Africa come to the forefront of public consciousness (43–45). Although conservation dog handlers do not perform the same tasks, comparisons can be made as both are at the front line of conservation. Improving employment conditions and ensuring adequate equipment and training were key recommendations following a survey of 570 rangers in 12 African countries (43, 44). Only one participant alluded to handler welfare when stating the importance of considering the handlers' welfare state in the handler-dog relationship. Although no other participants discussed this, it does not mean it was not considered important as the interview focus was on the health of the dogs.

The selection and training process was recognized as being key to the maintenance of the dogs' health. The selection of handlers was often from a cohort that had not had very much experience of working with dogs or of having a relationship with dogs which is commonly recognized in sub-Saharan Africa (46), so required a unique skill to recognize which handlers would be suited to the job. The correlation between handler-dog proficiency and handler personality, and the dog-handler relationship has been previously established (11, 38, 47–50). None of these studies are directly related to the recognition of health problems. In our study, training inexperienced handlers to ensure the optimum health of the dogs involved instructing them on routine health management such as grooming and recognizing signs of ill health and poor working conditions. Handlers were trained to prioritize dog health above performance. The handler-dog bond was allowed to develop by incorporating routines in which the handler-dog bond could be nurtured. This was directly linked to handler-dog team performance as without the dogs being healthy, they could not perform. Organizations wanting a successful conservation dog program must be aware of this vital step in its establishment.

Handler experience was not recognized as being important in the health of the conservation dogs. This is in line with a study examining factors affecting welfare of military dogs in Belgium (38), but in contrast with several studies on wildlife detection programs (11, 51). This highlights the importance of the initial selection and training process, but as most of the programs had not been running for a very long time (median 7 years) it could be that there were not enough experienced handlers for the benefit of their experience to be recognized. Also, there are no studies looking directly at canine health and handler experience, so without looking at this directly one can only speculate from these performance studies that health is incorporated in handler-dog performance. Future studies investigating the link between canine health and handler experience would be beneficial.

4.3. Evidence based approach to healthcare

The lack of evidence-based management of the most important health risks to the conservation dogs was a challenge to the health of the conservation dogs. Evidence based decision making is missing or inconsistent in the working dog field (52–54). In this study, lack of research on the management of health conditions specific to conservation dogs in Africa, lack of access to information including from competent veterinarians, a reliance on easily available information from the internet and other programs and a reliance on traditional management techniques all contributed to management that had a weak evidence base. For example, the use of stun guns to treat venomous bites and stings is a practice that has been discredited in the literature but is practiced by well-meaning conservation dog handlers (55). This is often the case when it comes to managing the health and welfare of working dogs, where small numbers and very specific tasks means that there is a lack of scientific research and a reliance on knowledge bases outside of science (12).

Programs were not able to confidently prevent and treat African canine trypanosomiasis due to the lack of research in this area. There was also concern about the long-term side effects of regular isometamidium injections, but there was no information on what these could be. The only studies so far investigating methods of prevention of trypanosomiasis is of a study on French military working dogs who had isometamidium administered every two to 3 months during their deployment to Ivory Coast or Gabon. Dogs deployed had a reduction in incidence of trypanosomiasis from 12.8% (19/148 dogs) to 2% at 0.5 mg/kg dosage (2/202) to 0% at 1.mg/kg

dosage. This supports the use of regular isometamidium injections, but this solitary short-term study does not evaluate the possible long term side effects. Studies on the longer-term effects of the drug would enable programs to make better decisions regarding its use. Understanding the pharmacokinetics of the drug would be a step toward this (56). There is also little evidence on how to prevent the acute adverse reactions that were reported. Intravenous administration is an effective treatment for trypanosomiasis in horses and avoids the muscular necrosis from intramuscular administration (56). This route of administration in dogs could be a way of avoiding side effects, which would be possible in an experimental trial but would need training in intravenous catheter placement and accurate dosing to limit potential toxicities (57). Regular screening as a means of detecting infection early, treating early and thereby reducing morbidity was reported to be successful by one of the participants who lived in an area with a high tsetse challenge (in combination with fly repellent strategies). The participant adopted this method following severe acute reactions in one of their conservation dogs and could also be investigated as an alternative to repeated prophylactic administration. The explanation of this method is an example of the knowledge exchange that Hall et al. described as being valuable (35). There is a clear lack of good quality studies investigating the prevention and treatment of canine African trypanosomiasis and further research is a priority.

The recognition, prevention and management of heatstroke has a considerable amount of published research but is deficient in terms of quality and relevance to the lifestyle of working dogs. Circular referencing and a lack of primary research has led to the establishment of guidelines and the implementation of practices that have a weak evidence base for preventing heatstroke in the working dog environment (58). Dogs are said to be suffering from heatstroke if their core temperatures are above 41°C with central nervous system dysfunction (59). However, a range of studies on dogs doing strenuous exercise range from 40 to 42.2°C without any adverse effects (58). Conservation dogs at extreme temperatures may regularly reach these body temperatures without ill effects, so relying on body temperature alone would be inadequate for considering whether dogs can work or not. Assessing changes in demeanor and thus preventing heatstroke and maximizing their working times is just as important if not more so than checking body temperature alone (58). However, if handlers are inexperienced, it may be safest to rely on numerical values rather than the handler's assessment of demeanor. Further research on the response of conservation dogs to working in high temperatures is required, so that handlers do not have to rely on poor evidence and evidence that is not specific to working dogs. A call for more research on the physiology of military working dogs and the best management methods for dogs working in extreme environments has already been made in this regard by Baker et al., and with the larger population of working dogs and availability of resources, hopefully this will come about. Conservation dog program workers and managers could apply this research if it were to be obtained either on their own or through their veterinarian, but like all good quality research it must be made readily available and not impede those who wish to access it by being behind a paywall (60). In conclusion, preventing and managing heat stress in conservation dogs should be individualized for each program depending on the population of dogs, the type of work and the experience of handlers.

Funding of research into the health of working dogs in conservation should be prioritized. Guidelines on best practice management of health in conservation dogs should be developed. This requires contemporary research to be available to handlers, support staff and veterinarians involved in conservation dog programs. Programs that pursue an evidence-based approach as best they can do well in advancing the health of their dogs (58). Training and improving accessibility of evidence-based protocols that are relevant for conservation dogs to both the staff of conservation dog programs and their veterinarians could improve this. Improving accessibility of evidence-based resources and education in evidence-based veterinary medicine to veterinarians working with conservation dogs. Working toward an American military working model where there are different stages of training in first aid, refresher courses and further training would mean that training could cater to every level of dog handler experience (21). Workshops like that conducted by Save The Rhino in 2018 and 2019, working with their veterinarians in learning first aid, onsite and online training are all ways that handlers could access support and further their skills (61). Providing veterinarians with access to courses in Evidence-based veterinary medicine, courses tailored specifically to treating working dogs and promoting the use of open-source literature will aid veterinarians to best support conservation dogs.

4.4. Conclusion

This is the first study examining the health of conservation dogs in Africa. The implementation of a conservation dog program faced multiple challenges from the acquisition of dogs, selection of handlers, training of handlers and dogs, health management, program management through to retirement. The resulting high investment into the dogs means they had high inherent value. Handlers form strong attachments to their dogs at an individual level. This was strengthened by working with the dogs in the challenging environment and seeing them succeed. Conservation dog programs should consider the fostering of the handler-dog attachment as a priority for the health of the dogs.

The high value of the dogs combined with the strong handler dog attachment meant that program staff were highly motivated to prevent, diagnose and treat disease. Program staff that worked with veterinarians valued their relationship highly, but some programs struggled to find appropriate veterinarians to work with. Providing training to veterinarians working with the dogs and using technologies such as telemedicine would improve access to quality veterinary care. Program staff found it difficult to find and evaluate evidence on best management practices, particularly when it came to preventing and treating trypanosomiasis. Further research into the prevention, treatment, and the effects of chemoprophylaxis of trypanosomiasis would go a long way to closing this evidence gap. Allowing access to the resources that result from any research into the health of working dogs in conservation and related fields should be prioritized such that program staff can work toward evidence-based health management.

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 studies involving human participants were reviewed and approved by Human Ethical Review Committee at The University of Edinburgh. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

NE was the principal researcher. NA was the principal project supervisor. JM provided her support with qualitative research approach. MP provided guidance using her expertise in working dogs in conservation in Africa. 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.2023.1179278/full#supplementary-material

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Biocidal effects of a wipe-down procedure using common veterinary cleansers on microbial burden within working canine exterior coats

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Introduction: Recent work demonstrating reduction of aerosolized contamination via a wipe-down procedure using common veterinary antiseptics offers promise regarding health concerns associated with cross-contamination from working canines to humans. While mechanical reduction can be achieved via a wipe-down procedure, the biocidal impact on flora within the exterior coat is unknown.

Methodology: This study assessed the biocidal impact of antiseptics on the exterior bacterial community of the canine. Lint-free towels were saturated with 2% chlorhexidine gluconate scrub, or 7.5% povidone-iodine scrub diluted at a 1:4 ratio. Treatments were rotated across the dorsal aspect of kennel housed Foxhounds ($n=30$). Sterile swabs were collected in triplicate prior to, and following wipe down, stored in Amies solution at 4°C, plated onto nutrient agar and reduction in colony forming units (CFU) was measured across both treatments. Statistical analysis utilizing PROC GLM examined effects of treatment ($p\leq0.05$). Molecular analysis of the 16S rRNA gene was completed for 3 hounds.

Results: Reduction in CFU was measured ($p<0.001$) for both antiseptics. Qualitative molecular data indicated that both antiseptics had a biocidal effect on the dominant microbial community on the exterior coat with gram-positive, spore-forming taxa predominating post-treatment.

Conclusion: Effective wipe-down strategies using common veterinary cleansers should be further investigated and incorporated to safeguard working canine health and prevent cross-contamination of human personnel.

KEYWORDS

canine, decontamination, wipe-down, biocidal, antimicrobial

1. Introduction

Cleaning strategies in healthcare facilities often include wipe-down procedures using detergents and disinfectants with biocidal activity to reduce microbial contamination of high-touch surfaces and prevent fomite transmission of pathogens to patients (2). As animal-assisted therapy for patients and healthcare personnel (HCP) has become more commonplace, the exterior coat of working canines represents a unique high-touch fomite surface not well-understood in infection prevention. While expert guidelines emphasize hand hygiene before and after each animal contact and describe how to prepare the animal prior to visiting a healthcare facility (e.g., bathe with a mild, hypoallergenic shampoo if malodorous or visibly soiled), recommendations regarding disinfection of the animal's coat between interactions with patients and HCP are lacking. Limited data are emerging regarding the efficacy of wipe-down procedures involving working dogs (including therapy and service dogs) (1, 3). However, the biocidal activity of these methods has not been well-characterized.

Outside of healthcare, working canines are frequently exposed to pathogens that can be harmful to the animal, its human handler, and others the animal may encounter. For example, disaster canines frequently deploy to contaminated environments with high levels of fecal coliforms (4, 5), often in the setting of compromised sewage systems (6). Recent emphasis on canine decontamination and hygiene has increased awareness of the risk of cross-contamination to humans (1, 7, 8). While canine-to-human cross-contamination with oil-based agents despite standard decontamination procedures has been described (8), the risk of microbial cross-contamination has not been well-characterized. Recent work identifying the shared microbiota of canines cohabitating with humans suggests such transfer is likely (9). Evidence-based canine decontamination strategies are needed to mitigate microbial contaminants present on the exterior coat of the working canine. Common bathing procedures utilized for canine decontamination are resource-intensive, impractical, and may result in damage to canine skin if repeated frequently (10, 11). A simple, practical wipe-down procedure would be useful in preventing fomite transmission of pathogens from the canine exterior coat to humans and their surrounding environment.

2. Materials and methods

2.1. Animals and swab collection

Institutional Animal Care and Use approval (# 19–031) was obtained from Southern Illinois University prior to the initiation of this study. Working canines (Foxhounds, $n = 30$) housed in similar outdoor kennel facilities were utilized in this study. Study participants included intact female ($n = 10$), intact male ($n = 11$) and neutered male ($n = 9$) dogs. All participants were considered "ideal" weight (BCS 4–5) and ranged from 2 to 11 years of age. Routine vaccinations as well as internal and external parasite prevention measures were current for all study participants.

Sterile cotton tipped swabs were utilized for sample collection. Swabs were collected following 30 s of contact time utilizing continuous bidirectional rotation while following the direction of coat growth. Swabs were collected in triplicate prior to and following wipe-down with one of two antiseptics evaluated and stored in Amies transport media (1 mM $MgCl_2$ x 6 H_2O, 1.5 mM KH_2PO_4, 8 mM Na_2HPO_4, 1 mM $CaCl_2$, 2.7 mM KCl, 50 mM NaCl, and 1 g sodium thioglycolate per L). Unused swabs were saturated in sterile deionized H_2O (prior to storage in Amies transport media) to serve as controls.

2.2. Wipe-down procedure

Disposable, lint-free towels (Davelen©; Derwood, Maryland) were saturated with 2% chlorhexidine gluconate scrub (CHX) or 7.5% povidone-iodine scrub (PVD) diluted in sterile water at a 1:4 ratio. The dorsal aspect of each canine was divided into left and right segments and treatment wipes rotated between left and right sides for each dog (see Figure 1).

Each canine was wiped down using CHX on one side and PVD on the other, alternating sides (left vs. right) with each successive participant. Wipe-down with the disinfectant-saturated towels was applied from the shoulder to the hip. Following this initial wipe-down, a second was applied in the same fashion using a water saturated towel to remove any disinfectant residue.

2.3. Bacterial analysis

Biocidal activity was measured quantitatively by colony count (colony forming unit, CFU). 0.1 mL Amies transport medium from each swab collection and transport device was inoculated onto nutrient agar (BD

FIGURE 1
Lateral view of PVD residue remaining after treatment wipe.

Difco™) plates using the spread plate technique. For swabs resulting in too many colonies to count (TMTC), serial dilutions were performed until a statistically significant number of colonies (30–300 CFU/mL) was obtained. Final colony counts for each of the triplicate swabs (including calculations for the diluted samples) were averaged.

In order to capture in depth microbial data, individual colonies isolated from swabs obtained prior to and following wipe-down for three dogs were submitted for standard polymerase chain reaction (PCR) targeting the bacterial 16S rRNA gene using the universal primers 8F (5'-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5'-GGTTACCTTGTTACGACTT-3′) (12) and DreamTaq (ThermoFisher), as described by the supplier. PCR cycling parameters consisted of an initial colony matter lysis step of 94°C for 10 min; followed by 30 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min; and ending with a final last extension step of 72°C for 10 min. Following agarose gel electrophoresis analysis, amplicons in the size range of ~1482 bp were extracted using the GeneJET (Thermo Scientific) gel extraction kit. The resulting purified DNA was then sent for commercial DNA sequencing using either 515F (5′- GTGCCAGCMGCCGCGGTAA-3′) or U529R (5′-ACCGCGGCKGCTGGC) primers, targeting regions V3-V4 of the 16S rRNA gene. A total of 144 partial 16S rRNA gene sequences were manually analyzed for purity and trimmed of primer sequences. From this initial analysis, 138 trimmed sequences (ranging between ~400–750 bp) were selected for BLASTn analysis (13). For each analyzed sequence, the BLASTn hit with the highest sequence similarity to a named isolate was recorded.

2.4. Statistical analysis

Data entry was performed using Microsoft Excel (Microsoft Corporation, Redmond WA) and data were analyzed using SAS, version 9.4 (SAS Institute Inc., Cary, NC). Significance for all variables of interest was established at $p < 0.05$. The effect of treatment was evaluated using a PROC GLM two-way ANOVA to identify changes in CFU count associated with PRE (untreated) versus POST (treated) counts for each cleanser utilized. Means and ranges of CFU values for PRE and POST values including percent reduction of treatments are reported.

3. Results

3.1. Bacterial quantification

Due to CFU counts exceeding the countable range, dilutions were performed to obtain values within the countable range (30–300 CFU/mL) for all PRE samples and 7 POST swabs from the CHX treatment (dog #7- all three swabs; dog #13- all three swabs; dog #30-one swab). No dilution was necessary for POST swabs collected after PVD wipe. CFU values for PRE samples ranged from 1.42×10^7 to 9.03×10^3 (mean: 1.80×10^6), while CFU values for POST CHX and PVD samples ranged from 1 to 5.16×10^5 (average of 3.16×10^4) and 6 to 113 (average of 32), respectively. The overall comparison of PRE values to POST values was highly significant ($p < 0.0001$) indicating efficacy of treatments. The CFU reduction for PVD was −99.98%, while the CFU reduction for CHX was slightly less at −98.61%. However, this difference between treatments was not significantly different ($p = 0.9192$; Figure 2).

3.2. Bacterial communities identified

An overview of bacterial taxa present on the external canine coat as well as species resistant to the biocidal activity of PVD and CHX wipe-down was determined using a molecular approach targeting the bacterial 16S rRNA gene in colonies obtained from canine participants #17, #19, and #21. Due to the significant biocidal activity observed with both antiseptics studied, few colonies were obtained from these samples (Figure 3).

Only 12 colonies per condition for each of the three canines were subjected to PCR targeting the 16S rRNA gene and subsequent sequencing. Because of the strong biocidal activity of CHX on canine

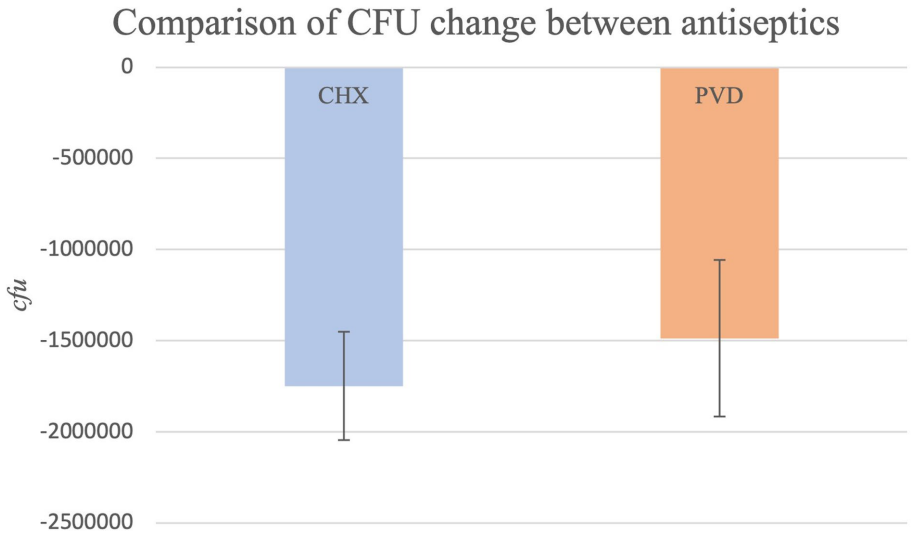

FIGURE 2
The comparison of mean CFU reduction for both antiseptic treatments ($p = 0.9192$).

#21, extra plating had to be performed to obtain 12 colonies for 16S rRNA gene analysis. The raw sequence analysis from the analysis of 138 sequences (6 sequences were not of good quality and removed) can be found in Supplementary Material. An overview of the orders detected in the swab samples for the three dogs are presented in Figure 4.

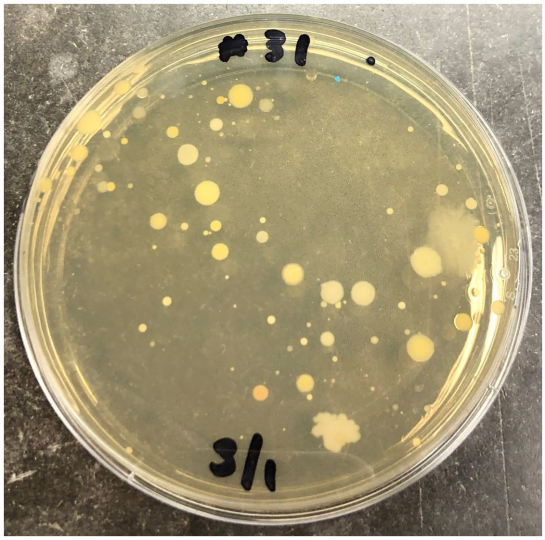

FIGURE 3
Undiluted sample from swab #31 corresponding to PVD treatment of canine #3 plated on nutrient agar.

Although gram-negative bacteria of the class Gammaproteobacteria were identified in PRE samples from all three canines, both PVD and CHX wipe-down effectively eliminated these taxa. In contrast, POST wipe-down samples for both PVD and CHX were dominated by members of spore-forming Bacillales. This order was detected at a much lower percentage from PRE samples (7.4% for participant #17, 12.5% for participant #19, and undetected for participant #21; Figure 4 and Table 1).

For a more specific analysis of relative abundance at the genus level, the sequence analysis of PRE and POST samples from dogs selected randomly (#17, #19, and #21) was combined (Figure 5). This analysis indicated that PRE samples were dominated by members of the gram-negative genus *Psychrobacter* (~43.7% relative abundance), a psychrotolerant (cold temperature tolerant) bacterium. No traditionally pathogenic genera were identified in PRE samples; *Staphylococcus* sequences detected were most closely related to the *equorum* species (~15.5% relative abundance; Figures 4, 5 and Table 1). Bacterial communities identified in POST PVD and CHX wipe-down samples possessed similar profiles in that gram-positive spore formers predominated (~80.6% relative abundance of *Bacillus*, *Fictibacillus*, *Deinococcus*, *Domibacillus*, *Lysinibacillus*, *Paenibacillus*, *Priestia*, *Psychrobacillus*, *Sporosarcina* and *Virgibacillus* species; Figure 5). *Bacillus* and *Priestia* species detected were non-pathogenic: *thuringiensis, altitudinis,* and *megaterium* (Table 1). Only one colony from the POST CHX wipe-down samples was related to a gram-negative species (*Rhodopseudomonas*).

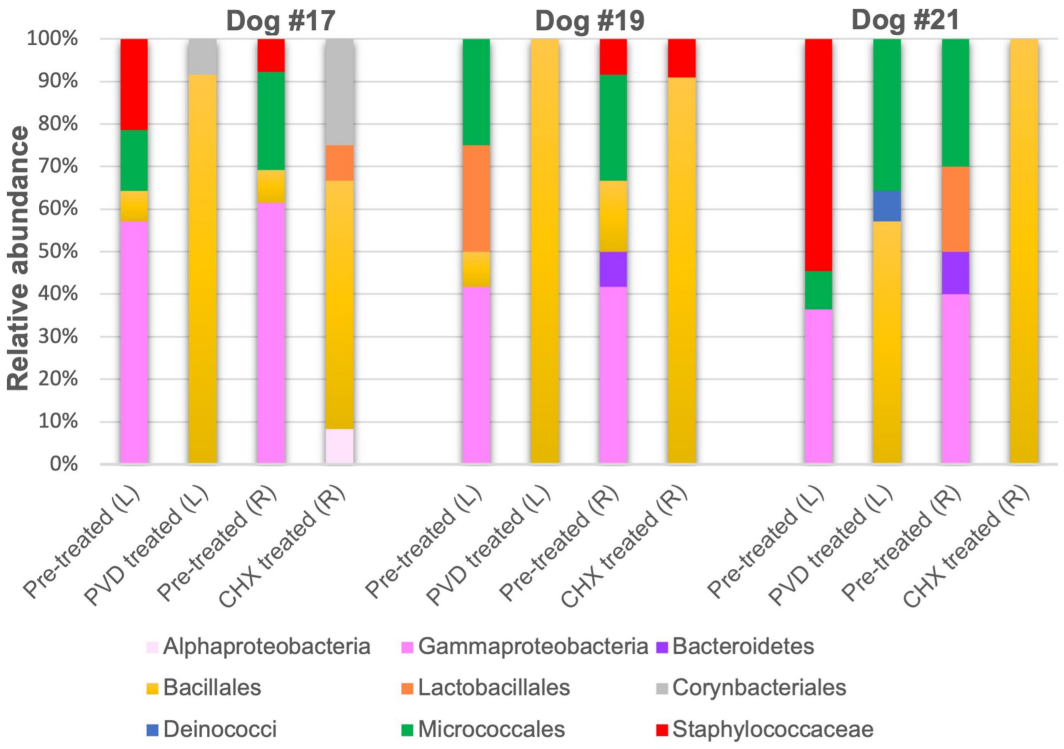

FIGURE 4
Relative sequence abundance of bacterial orders determined from 16S rRNA gene analysis of colony forming units. PVD: 7.5% povidone-iodine, CHX: 2% chlorhexidine, L: left side, R: right side.

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4. Discussion

A simple disinfectant wipe-down procedure using towels saturated with 2% chlorhexidine gluconate scrub or 7.5% povidone-iodine scrub exhibited significant biocidal activity against bacteria present on the exterior coat of working canines, resulting in 99.98 and 98.61% reduction in CFU, respectively (Figure 2). Molecular characterization of a smaller subset of samples demonstrated shifts in bacterial community composition after wipe-down with CHX or PVD. No pathogenic bacteria were identified. These findings suggest that wipe-down procedures using CHX or PVD are effective in reducing microbial burden and exert selection pressure on resident bacterial flora present on the canine exterior coat.

While a limited analysis of the microbial community present on the external coat of three canines was performed, the microbiome selected for further analysis prior to disinfectant wipe-down was dominated by *Psychrobacter* species (Figure 5; Table 1). *Psychrobacter* are psychrophilic or pyschrotolerant (cold-loving or cold-tolerant) gram-negative bacteria associated with a wide range of animals as well as terrestrial and marine environments. *Psychrobacter* species have recently been detected among canine oral (14), conjunctiva (15), and skin (16) microbiomes. Species within this genus rarely cause disease (17) and were likely selected for due to the winter season in which the samples were collected. It should also be noted that the swabs were stored at 4°C post collection.

Another genus of interest detected in samples obtained prior to disinfectant wipe-down was *Staphylococcus* (Figure 5). However, none of these colonies were closely related to pathogenic *Staphylococcus* species (Table 1). Most of the *Staphylococcus* sequences were most closely related to the *equorum* species, which have been isolated from healthy Labrador

TABLE 1 Taxonomic assignment of most abundant bacterial 16S rRNA gene clone sequences.

Order	Genus/species	GenBank Accession/ average % identity match	% of sequences per treatment per animal[a]		
			#17	#19	#21
Pre-treated assignments					
Micrococcalles	*Glutamicibacter bergerei* HMF3875	KT983988.1/100%	–	–	14.3
Micrococcalles	*Glutamicibacter species* IR2A07	MK841225.1/99.8%	3.7	13.0	4.8
Lactobacillales	*Carnobacterium inhibens* 96E2	MT032346.1/99.3%	–	13.0	–
Staphylococcaceae	*Staphylococcus equorum* JZ RK-17	MH119700.1/100%	–	–	9.5
Staphylococcaceae	*Staphylococcus equorum* LS220	MT409914.1/100%	11.1	–	14.3
Gammaproteobacteria	*Psychrobacter faecalis* NC7 16S	MT269580.1/99.9%	29.7	26.1	4.8
Gammaproteobacteria	*Psychrobacter maritimus* JM52	MN758812.1/99.9%	11.1	–	–
Gammaproteobacteria	*Psychrobacter submarinus* QS172	MK439598.1/100%	14.8	4.3	–
Post-treated assignments					
Micrococcalles	*Kocuria palustris* RP1	MH141481.1/99.1%	–	–	8.0
Corynbacteriales	*Rhodococcus equi* p67_A11	Q831084.1/99.5%	8.3	–	–
Bacillales	*Bacillus altitudinis*	MT627439.1/100%	–	11.1	–
Bacillales	*Bacillus thuringiensis* GR007	CP076539.1/99.6	–	-	8.0
Bacillales	*Paenibacillus* species 7B-648	KF441697.1/99.2%	–	11.1	–
Bacillales	*Priestia megaterium* FORCN119	MW363319.1/100%	12.5	11.1	–
Bacillales	*Priestia megaterium* S2	CP051128.1/99.8%	8.3	–	–
Bacillales	*Priestia megaterium* UIS0181	MT178181.1/100%	16.7	5.6	–
Bacillales	*Sporosarcina globispora* LMTK33	KY614182.1/99.8%	–	–	16.0
Bacillales	*Sporosarcina* species A9	MN746652.1/99.6%	–	–	12.0

[a]Relative abundance of specific sequences detected at >5% for at least one animal.

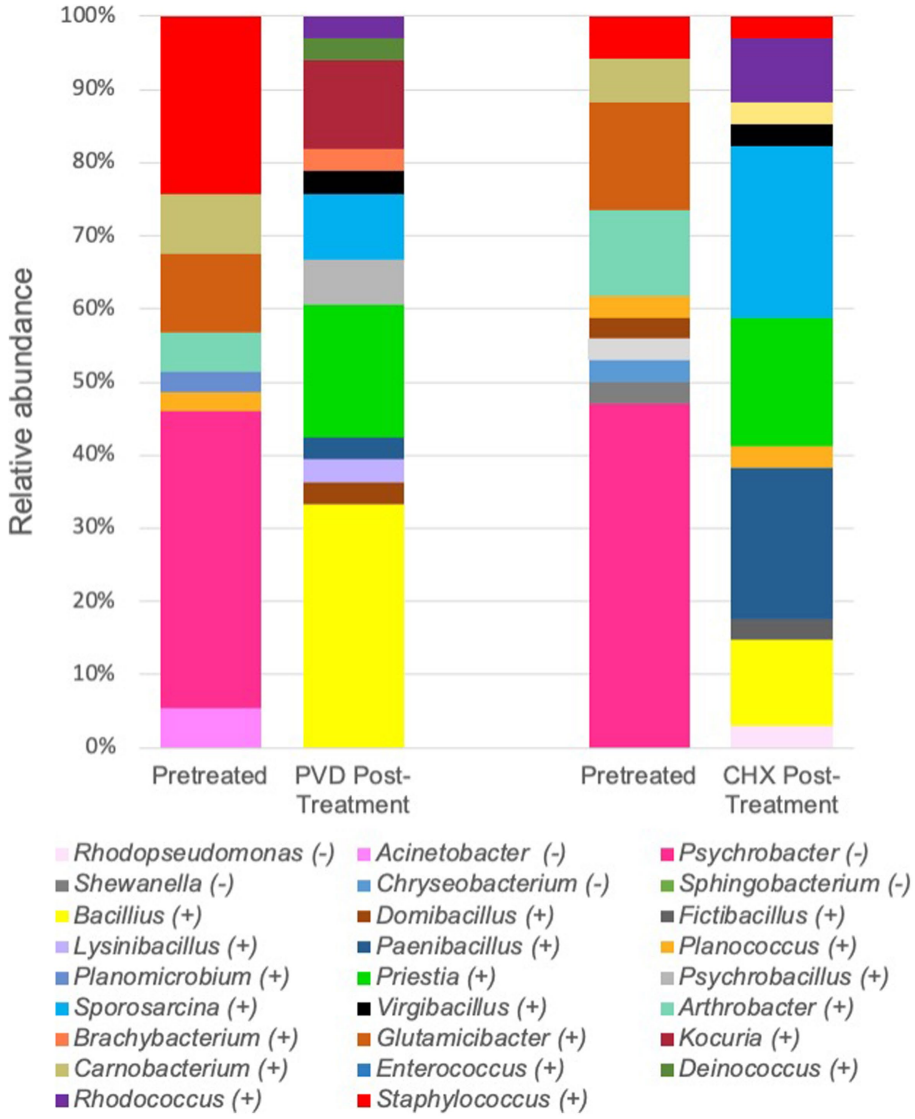

FIGURE 5
Combined relative sequence abundance of bacterial genera determined from 16S rRNA gene analysis of colony forming units. PVD: 7.5% povidone-iodine, CHX: 2% chlorhexidine, (–): gram-negative, (+): gram-positive.

retrievers (18). The qualitative results from the pre-wipe samples suggest that the canines possessed similar external coat microbiomes, likely a reflection of an environmentally homogenous study cohort.

Samples obtained after disinfectant wipe-down were dominated by the non-pathogenic aerobic/facultative spore-forming gram-positive genera of *Bacillus*, *Paenibacillus*, *Priestia*, and *Sporosarcina* (Figure 5; Table 1). Specifically, *Priestia* (formally *Bacillus*) *megaterium* and *Sporosarcina globispora* were two detected species that are commonly found in the environment (19). While it has been reported that PVD is more sporicidal than CHX (20), these studies were focused on spore formation in *Bacillus subtilis*. No CFU related to the gram-negative *Psychrobacter* species identified in the pre-wipe samples were detected in the either the PVD or CHX treated samples (Figure 5). However, our study design provided only a snapshot of the external coat microbiome and was not able to determine if bacterial species were differentially targeted by PVD and CHX wipe-down. A more exhaustive microbial community analysis is necessary to determine the bactericidal spectrum of each disinfectant on flora present in the canine exterior coat.

The implications of this work to the working canine community are significant. The benefits of working canines across many disciplines is well documented, including in healthcare settings (21, 22). However, the potential for canine-to-human microbial cross-contamination remains an important concern in infection prevention and evidence-based canine decontamination and hygiene procedures are needed. Therapy dogs, service dogs, law enforcement dogs, and disaster dogs are frequently tasked with work resulting in a high degree of contact with the environment which can lead to human cross-contamination with both canine and environmental flora. Prior work has demonstrated that a simple wipe-down procedure utilizing CHX is effective at reducing exterior coat contamination with aerosolized contaminants (1). More recent work investigated the potential efficacy of CHX as a wipe down decontaminant for canine equipment with significant success using viral surrogates (23).

This work clearly demonstrates a beneficial reduction in canine coat microbial burden following a simple wipe-down procedure. Kennel-housed dogs were utilized in this study in order to identify

potential benefits in dogs with outdoor exposure. Future studies should incorporate hospital-based working canines in order to assess impacts to microbes typically present in a hospital environment. Additionally, dogs of different breeds with differing coat types should be assessed for potential differences due to coat morphology.

5. Study limitations

Disruption due to the COVID 19 pandemic resulted in lack of access to a larger study population due to travel limitations for study technicians. Future work should include breeds commonly utilized in working disciplines including service and therapy dogs. Additionally, future work should include microbial analysis from various anatomical regions to capture a more comprehensive picture of the entire dermal environment and impacts associated with bathing.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/; OR174799 - OR174935.

Ethics statement

The animal study was reviewed and approved by the Southern Illinois University Institutional Animal Use and Care Committee. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

EP supervised study design, study execution, data collection, data analysis, manuscript writing, and review. DD, KW, and KB contributed to study design, study execution, data collection, data analysis, manuscript writing, and review. SL contributed to study design, data analysis, manuscript writing, and review. 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.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2023.1219249/full#supplementary-material

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Impacts to canine dermal microbiota associated with repeated bathing

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Introduction: Working dogs routinely operate in environmental conditions which may necessitate daily bathing to remove contaminants or soilage. The impacts of frequent or repeated bathing on the canine dermal microbiota are unknown. The objective of this study was to characterize changes in canine dermal microbial populations following repeated daily bathing.

Methods: Labrador retrievers (n = 16) were bathed daily using a dilute dish detergent solution (1.6% detergent solution) over the course of 14 days. Dermal microbial DNA was collected *via* sterile swabs (n = 142) taken at days 0, 7, 14, 16, 21, 28, 35, 42, and 49 and analyzed for alpha diversity, beta diversity and relative abundance to assess changes in the dermal microbiota *via* 16 s sequencing.

Results: Results indicate that daily bathing significantly increased Shannon diversity, Chao1, and several rare amplicon sequence variants. Although typically reported in highest abundance, relative abundance was decreased in the phyla Actinobacteria, Firmicutes, and Proteobacteria ($p < 0.05$).

Conclusion: Repeated daily bathing with dilute dish detergent significantly reduced normal healthy dermal microbial taxa and created significant changes in the dermal microbiota of canines. Disruption to the canine dermal microbiota may cause negative impacts to canine dermal health and require further investigation.

KEYWORDS

working dog, decontamination, dermal microbiota, canine, bathing

1. Introduction

Current research on the microbiota of the canine dermis has focused on pathologies such as allergies and atopic dermatitis. Previous reports have characterized relative abundance of predominant taxa in both healthy and diseased populations (1–4). Several studies have reported Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria in highest abundance (1–4). Many factors may impact the resident dermal microbiota. Atopic dermatitis has been reported to cause significant shifts in microbial composition (1, 5).

Prior efforts aimed at identification of factors impacts dermal microbiota have reported individual variation as the largest contributor to microbial change (4). Other factors include anatomical location of sample and breed. In addition, dietary influence has also been identified as a source of potential impact on dermal microbiota (6). Other factors that may be disruptive to dermal microbiota include topical treatments such as the use of cleansers or detergents.

Exposure to detergents has been reported as a contributing factor leading to dermal microbiota disruption in humans, likely due to altered cutaneous pH (7–10). Dermal irritation has also been associated with altered pH (11, 12). However, the impact of cleansers and detergents on canine dermal microbiota is currently underrepresented in the scientific literature.

Although changes in resident dermal taxa may be of consequence to the canine, there are potential effects for their human counterparts as well. Previous reports have demonstrated that humans in contact with canines develop shared dermal and intestinal microbiota (13–15). Canines and other household pets may act as a fomite and have been reported to carry pathogenic bacteria including *Staphylococcus* (16–19). *S. pseudintermedius,* a resident of the canine dermal microbiota, can colonize human skin potentially causing infection (17). The incidence of infection in humans is not well known as the infection is frequently assumed to be *S. aureus,* which in some cases can also be passed from canine to human (20). Additionally, specific strains of *S. pseudintermedius,* as well as *S. aureus*, are multi drug-resistant which complicates treatment (18, 20). Therefore, factors which increase the abundance of these potential pathogens increase health risks and should be avoided. Pathogens colonizing the dermal microbiome present potential risks to human handlers.

Working canines frequently live and travel in close proximity to their handlers and teams, increasing the potential for spread of pathogenic bacteria should they be colonized. Additionally, working canine management includes frequent use of detergent as part of required decontamination protocols due to the high likelihood of contaminant exposure at deployment areas such as disaster sites (21) or urban environments (22, 23). This recommendation, however, fails to consider that search and rescue (SAR) canines may be deployed to disaster sites for up to fourteen consecutive days requiring daily decontamination(s). Prior assessments from deployed canines responding to the Oso, Washington mudslides reported skin irritation in canines decontaminated daily with a dish detergent within 3 days (24). It is possible that these decontamination procedures may result in shifts of the dermal microbiota which is frequently associated with dermal irritation. The reported symptoms diminished after cessation of detergent usage.

Data is needed to clarify the impacts of daily use of detergent on dermal microbiota as well as its ability to recover once bathing has ceased. Therefore, the objective of this work is to identify the changes in dermal microbiota associated with daily decontamination utilizing a dish detergent and characterize the recovery of the dermal microbiota following cessation of decontamination.

2. Materials and methods

2.1. Animals and treatments

This research was approved by the Southern Illinois University Institutional Animal Care and Use Committee (15–032) as well as by the Royal Canin ethics committee. Labrador retrievers ($n = 16$) from a research colony were utilized for this study. Labrador retrievers were selected as they are both a popular working and pet breed. Exclusion criteria included use of medications such as antibiotics, history of allergies, and history of dermatological conditions. Canines were housed in their resident kennel environment with two canines per indoor/outdoor run. All canines were up to date on regular monthly parasite control as well as standard vaccinations. Facilities were equipped with an on-site veterinary team in the event of any discomfort or illness in the study population. One canine developed atopic dermatitis on day 14 on the point of hip and in accordance with IACUC guidelines and veterinary team recommendations, was removed from further testing to pursue treatment. Canines were fed a chicken-based diet which was formulated to meet or exceed the NRC requirements with 21% protein and a minimum 10% crude fat. Canines had a minimum of 90 days acclimation to the diet prior to the initiation of the study.

All canines received simulated decontamination once daily for 14 consecutive days. The study protocol was adapted from previously published recommendations working canine decontamination methods (25). Decontamination was carried out by trained technicians with controlled water temperature settings (approximately 36C°), washing order, washing pressure, and rinse times. Decontamination began with total body saturation using a spray nozzle applying water from the base of the neck to the tip of the tail. 16 oz. of dilute dish detergent solution (Dawn® dish detergent, Proctor & Gamble, Cincinnati, OH; diluted 59 mL of detergent to 3.7 L of water) was applied evenly across the shoulders, back, ribcage, chest, abdominal area, and legs. The shoulders, back, left side, right side, chest, and abdominal area of the canine were massaged to a lather. Dorsal and ventral anatomy received a lathering massage for 2 min across each area. The legs were lathered and washed for 30 s each. Each canine was rinsed until no soapy residue remained (approximately 4–5 min). Canines were towel dried using two separate clean towels. The canine coat was left damp but not saturated. All towels were 100% cotton and of identical make and model series (Towelhub®, Atlanta, GA).

2.2. Data collection and visual assessments

Dermal swabs and visual assessments were collected on days 0, 7, 14, 21, 28, 35, 42, and 49 by a single trained technician. Collection on day 0 occurred prior to the commencement of decontamination. Dermal microbiota swabs were taken from a $3cm^2$ area on the point of the right hip using the Norgen Biotechnology (Ontario, Canada) swab collection and total DNA preservation system utilizing 30 s of skin contact with continuous rotation. In accordance with manufacturer recommendations, collection sites were shaved using a #40 blade (Oster, Boca Raton, FL) following the direction of hair growth prior to each data collection. An additional dermal microbiota collection was added on day 16 to observe changes 48 h following the last decontamination.

Skin health scoring assessments were performed by two trained technicians (see Supplementary material 1). Coat shine, coat condition, back dander and body dander were scored as adapted from previously published works (11, 26, 27). All visual assessments were conducted on a 0–4 (lowest to highest) scale with half point increments acceptable.

2.3. Microbiota analysis

Microbial DNA from swabs was extracted at Southern Illinois University utilizing the Norgen Microbiota DNA Isolation Kit

(Ontario, Canada). Isolated DNA was submitted for next generation sequencing (Diversigen Inc.) *via* 16S amplicon sequencing pipeline. Low DNA concentrations resulted in the removal of 8 samples. Thus, data reported here include 134 dermal microbial samples. Variables of interest include differences associated with study day, coat shine, coat condition, body dandruff, and back dandruff.

2.4. Alpha diversity analysis

Analysis of alpha diversity of canine dermal microbiota was carried out using amplicon sequence variants (ASVs). Rare ASVs, present in less than 10% of the samples, were removed from the ASV table resulting in a total of 1,530 ASVs. Three different alpha diversity metrics, Shannon, Chao1, and Observed ASVs, were calculated from the filtered ASV count table rarefied to the minimum sequencing depth using the vegan package in R. To determine whether daily bathing affects alpha diversity, a linear mixed model including day as a fixed effect and dog identity as a random effect was utilized [alpha diversity ~ day + (1|dog name)]. Additionally, a linear mixed model including day, skin and coat condition scores, and their interaction as fixed effects and dog identity as a random effect [alpha diversity ~ day * variable + (1|dog name)] was constructed and utilized to identify any confounding effect on daily bathing.

2.5. Beta diversity analysis

Beta diversity analysis was carried out using three different distance methods: Bray-Curtis, Unweighted UniFrac (considers only presence absence), and Weighted UniFrac (accounts for abundance of taxa). To determine the impact of study day on beta diversity, a Principal Coordinates Analysis (PCoA) plot was generated including all samples colored by days (Figure 1) Additionally, the envfit function from the vegan package in R was used to assess changes to beta diversity across study days as well as effects associated with skin and coat condition scores (Figure 2).

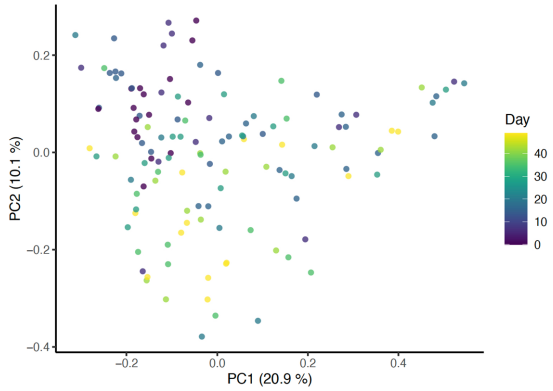

FIGURE 1
PCoA of Bray Curtis distance colored by study day ($p = 0.001$, $R^2 = 0.27$).

2.6. Differential abundance

Differential abundance analyses were performed across all taxonomic levels, including Phylum, Order, Family, Genus, ASV. To account for compositionality of microbiota data, the raw counts of taxa were transformed to Centered Log Ratio (CLR)-transformed abundance distributions using Monte-Carlo (MC) sampling (N = 20 instances) as implemented in the ALDEx2 R package. To assess changes to taxa abundance related to daily bathing, a linear mixed model was created including day as a fixed effect and dog identity as a random effect for each taxon on each MC instance of CLR-transformed abundances: taxon ~ day + (1|dog name). Additionally, a linear mixed model including day, skin and coat condition scores, and their interaction as fixed effects and dog identity as a random effect [taxon ~ day * variable + (1|dog name)] was constructed and utilized to identify any confounding effect on daily bathing.

Raw value of ps were collected within each MC instance and were corrected for multiple hypothesis testing (testing multiple taxa) using the Benjamini-Hochberg (BH) method. The average expected value of p across all MC instances was calculated for both raw and BH corrected *p*-values and reported as the final result. For any significant taxa, scatter plots of CLR-transformed abundance of taxa over time with an overlaid smooth line from LOESS regression were generated. In addition, if a taxon was significantly associated with a visual assessment, boxplots/strip charts of that variable were created (Figure 3).

3. Results

3.1. Alpha diversity

Results show that daily bathing increased Shannon diversity ($p < 0.001$), Chao1 ($p < 0.001$), and number of ASVs observed ($p < 0.001$) throughout the study (Figure 4). Alpha diversity for each measure peaked at day 35. Coat condition impacted Chao1 index ($p = 0.018$). Chao1, which measures the richness of the samples, increased at the lowest coat condition score (score = 1). This reveals an inverse relationship between species richness of the dermal microbiota and coat condition score. Additionally, coat dander impacted Chao 1 ($p = 0.039$) with Chao 1 lowest at dander score of 1 and highest with a score of 2 (Figure 5). As dander scores increased, species richness increased, but as coat condition improved (and scores increased) richness decreased. Coat condition and back dander changes did not interact with the changes associated with study day changes.

3.2. Beta diversity

Presence of taxa was impacted by daily bathing as unweighted UniFrac distances were significantly different across days ($p = 0.001$, $R^2 = 0.27$). As expected, samples from the same dog showed greater similarity when compared to samples from other dogs ($p = 0.001$, $R^2 = 0.31$) (Figure 6). When taxa abundance was considered, weighted UniFrac distances were unaffected by day ($p = 0.851$, $R^2 = 0.00266$) but remained clustered by dog ($p = 0.001$, $R^2 = 0.345$). These data indicate

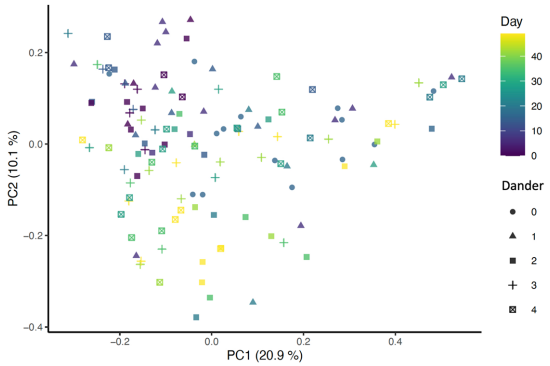

FIGURE 2
PCoA of Bray-Curtis distance colored by day ($p = 0.001$, $R^2 = 0.27$) and shaped by dander score ($p = 0.025$, $R^2 = 0.0708$).

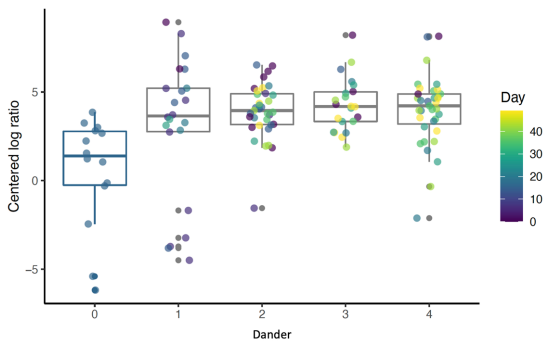

FIGURE 3
Boxplot showing abundance of Cyanobacteria for different levels of back dander ($p < 0.001$) by day ($p = 0.69$).

that the abundance of taxa were not impacted by daily bathing, only the presence or absence of specific taxa.

Significant associations between beta diversity metrics and coat condition, coat shine, and back dander were also found indicating each variable contributed to differences between the samples. Using the Bray-Curtis distance, coat condition ($p = 0.052$), coat shine ($p = 0.004$), and back dander ($p = 0.025$) (Figure 2) were associated with changes to the dermal microbiota composition. When controlling for these variables within the statistical model, the difference in the microbial composition between days remained significant ($p = 0.001$, $R^2 = 0.27$) (Figure 1), Accordingly, Bray-Curtis distance effects appear to be independent of any effects related to study day.

3.3. Differential abundance

Daily bathing significantly decreased the relative abundance of commonly predominant bacteria including Actinobacteria ($p < 0.001$), Firmicutes ($p < 0.001$), and Proteobacteria ($p < 0.001$) (Figure 7). Additionally, the abundance of 60 taxa at the genus level were significantly changed by daily bathing. The 25 most abundant are shown in a heatmap (Figure 8). Cyanobacteria were significantly related to dander ($p < 0.001$) but not day ($p = 0.69$). Additionally, there was no interaction for changes in Cyanobacteria for day and dander scores ($p = 0.856$). However, it is important to note that abundance of Cyanobacteria increased with increased dander scores (Figure 3).

4. Discussion

Improved understanding of the canine dermal microbiota is an important area of research and is a crucial component of canine dermal health. Prior reports of taxa present have focused on organisms associated with disease states such as atopic dermatitis (AD) (5). While prior decontamination recommendations have included the use of dish detergent or an alkaline cleanser for working canines receiving decontamination daily (25), the data presented here challenges those recommendations. Moreover, this is supported by the skin irritation documented following daily decontamination with dish detergent on dogs during a deployment (24). The data presented here provide additional evidence regarding changes to the dermal microbial composition associated with prior recommendations for the use of dish detergent.

Dermal microbiota studies in canines experiencing AD have reported key differences between populations of canines with healthy skin and populations of canines with AD (1, 3, 5, 28). Overall, canines who are healthy and canines with AD have the same general taxa present, but the abundance of that taxa differs by health status (1, 3). Canines from these studies are reported to have predominant phyla including Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, and Cyanobacteria level (1, 2, 4). However, contradictory findings were reported in canines suffering from AD with an overall lower number of observed taxa present than healthy dogs (2). These differences may be explained by different coat types, anatomical locations, or varying study population breeds. Nevertheless, the prior studies consistently agree that relative abundance is associated with AD and some key taxa become more abundant when compared to healthy controls.

Similar to prior work, this study identified Proteobacteria, Firmicutes, and Actinobacteriota as the dominant phyla (1, 2, 4). Daily bathing study affected microbial composition as unweighted UniFrac distances were significantly grouped across study day. Furthermore, daily bathing significantly increased Shannon diversity, Chao1, and number of ASVs observed. These findings indicate that species richness as well as abundance of those species differed throughout the study, with greatest impacts observed during bathing the active bathing period. Diversity increased following cessation of bathing and continued throughout the study timeline but failed to return to baseline measures. These results may indicate increased species richness, as well as increased abundance of those species in the dermal microbiome brought on by repeated bathing with dish detergent solution.

It is interesting to note that based on the trends of the presented results, the lowest measures of alpha diversity were observed on day 7. This trend may indicate that the negative impacts to the resident taxa had already occurred after a single week of daily decontamination with very dilute dish detergent solution. However, additional testing is required to further understand the rate at which dermal microbiota becomes compromised and at which it may also recover.

Reported changes in alpha diversity were accompanied by changes in relative abundance of the most predominant dermal

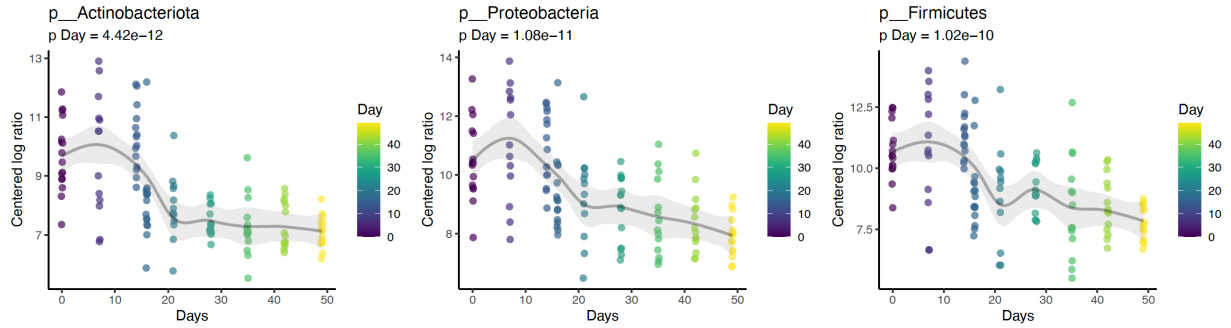

FIGURE 4
Daily bathing significantly increased Shannon Diversity Index ($p < 0.001$), Chao1 ($p < 0.001$), and number of ASVs observed ($p < 0.001$).

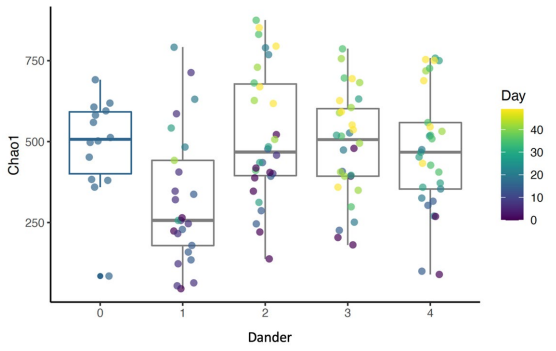

FIGURE 5
Boxplot showing Chao1 index for different levels of back dander ($p = 0.0389$).

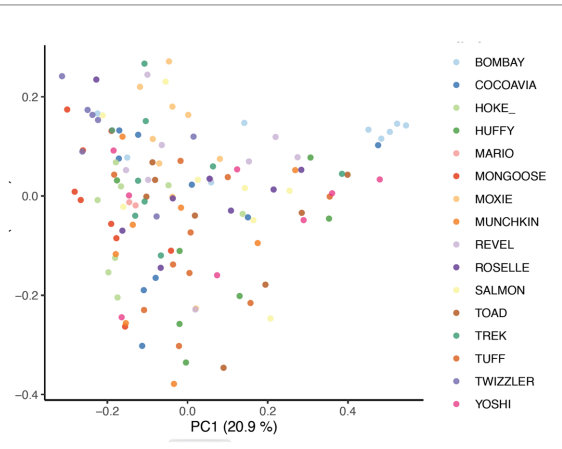

FIGURE 6
PCoA of Bray-Curtis distance colored by dogs ($p = 0.001$ $R^2 = 0.31$).

microbiota during repetitive daily bathing, with decreases in abundances of these taxa below original baseline measurements throughout the study. These findings, along with the changes in alpha diversity indexes, confirm that serial bathing had lasting impacts to the dermal taxa of the canines. However, it is currently unknown whether these changes are beneficial or not. It is possible that the bathing treatment decreased the abundance of the most dominant taxa allowing the non-resident taxa to utilize more resources and increase in presence. This theory may be supported by decreases in alpha diversity observed at day 7. The predominant phyla in this study (Proteobacteria, Firmicutes, and Actinobacteriota) peaked in abundance at the 7-day mark, after which their abundance decreased, potentially giving rise to increased species richness. Increased richness may not be beneficial in all instances. It is possible that these changes may lead to atopic dermatitis as suggested previously (1–3). Additionally, there is a possible risk associated with increased colonization of zoonotic pathogens present within the environment where the canine may live and or work.

It is unclear from prior studies whether changes in taxa presence and abundance are a result of dermal disease through decreased nutrient availability (water and lipids) or by disrupted dermal barrier integrity. However, authors have concluded that changes in the microbial profile are an indication of changes in dermal health (29, 30). Further work should better identify the relationship between the dermal microbiota, skin disease and barrier disfunction through measurements like trans-epidermal water loss (TEWL) and cutaneous pH (31, 32).

Several studies mentioned above have found that increased abundance in *Staphylococcus* is primarily associated with AD in canines (2, 3, 5). The data presented here demonstrate a significant increase in the abundance of *Staphylococcus* that peaks during the time of serial bathing and then falls below baseline values when daily bathing ceases. This finding may indicate that repetitive, daily bathing of dogs with dish detergent increases the risk for AD or infection. The increased risk may be a result of increased moisture exposure to the skin as well as cleanser effects to dermal pH which may facilitate a preferential environment for *Staphylococcus* to thrive. Although this work does not report beyond genus, should these increased *Staphylococcus* species be potential pathogens such as *S. pseudintermedius* or *S. aureus*, findings could indicate increased risk to handlers and teammates. Future work should identify if the changes in *Stapylococcus* by species associated with repetitive bathing give rise zoonotic pathogens as described.

Prior studies have identified a potential relationship between coat condition scores and dander with skin health (27, 33, 34). The findings presented here provide additional evidence for the relationship between dermal microbiota and coat condition and

dander. These findings are novel and offer further evidence that the dermal microbiota plays a crucial role in dermal health. Additionally, these data suggest that the association between visual characteristics and the dermal microbiota based on significant changes in beta diversity using the Bray-Curtis distances. These differences indicate that the visual assessments accounted for a significant amount of the difference between samples when taking into consideration both presence of the taxa as well as the relative abundance. Alpha diversity values of species richness (Chao 1) were significantly different by coat condition in addition to dander. This indicates that as dander becomes more evident (scores increased) species richness decreased, but as coat condition improved (and scores increased) species richness decreased. These are contradictory findings; however, it may be possible that increased lipid production as part of dermal healing may have had an effect on coat condition (35). Lastly, the abundance of several individual taxa changed in conjunction with visual assessments. One example is the change in Cyanobacteria in relation to dander. The abundance of Cyanobacteria increased as dander score increased. The increased abundance of Cyanobacteria is of particular concern due to the cytotoxins (known carcinogens) which are created as secondary metabolites (36). Further research should examine individual taxa changes and potential relationships to changes in coat and dermal health.

It is evident that the serial decontamination of the canines in this study led to lasting changes in the dermal microbiota of the canines. Previous work has demonstrated that the dermal microbiota extends past the surface of the epidermis into the dermis (37), and therefore future work on decontamination and bathing practices should investigate the effects of the dermal microbiota past the surface of the epidermis. Other studies have noted that taxa composition and abundance may be altered by anatomical location. Future work should also sample from various anatomy to identify potential differences (1).

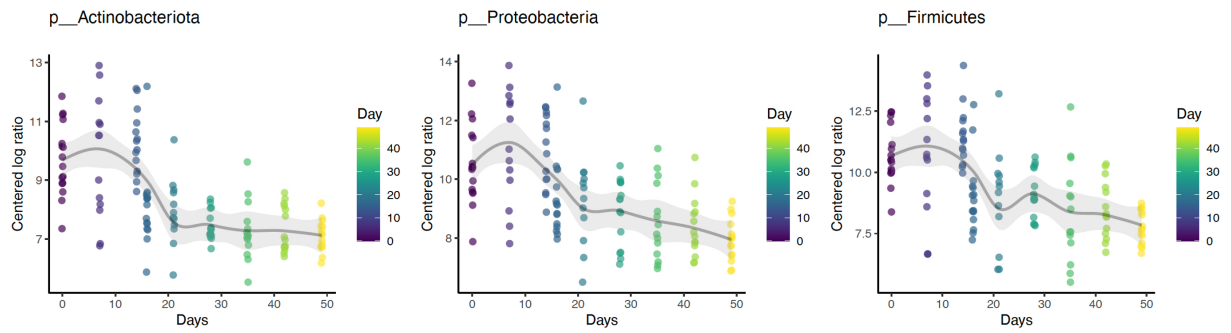

FIGURE 7
Daily bathing decreased the abundances of taxa Actinobacteria ($p < 0.001$), Firmicutes ($p < 0.001$), and Proteobacteria ($p < 0.001$) at the Phylum level.

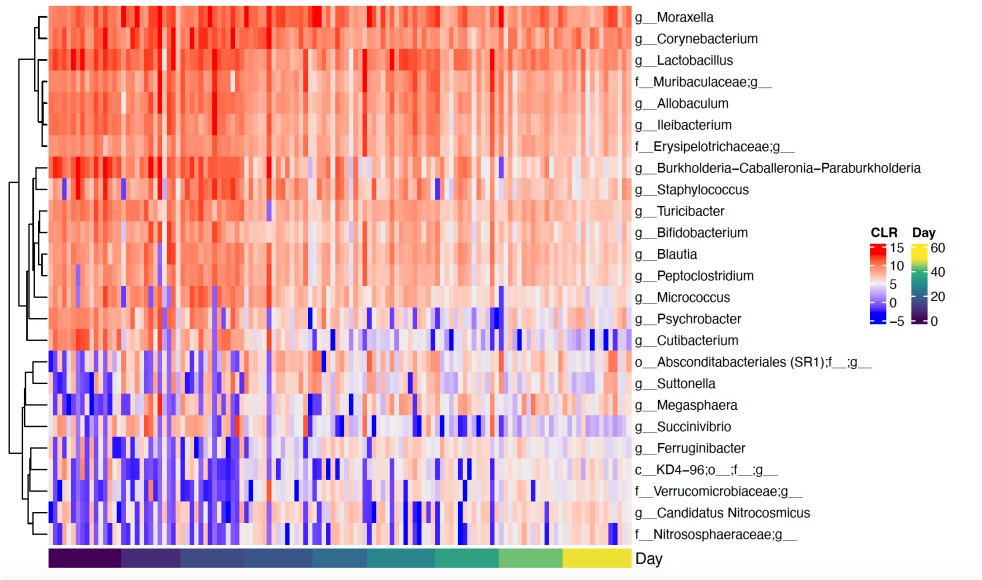

FIGURE 8
Heatmap showing CLR-transformed abundances of the top 25 most abundant genera that were significantly changed over time. If a genus is unclassified, its higher taxonomic level is shown in the heatmap.

Although the full nature of the relationship between dermal microbiota and dermal health is unknown, the changes in dermal microbiota associated with dandruff occurrence is another finding which appears to support a close relationship. It is also important to note that the cleanser utilized in the decontamination protocols employed for this study is more dilute than that typically used in the field. Dish detergent utilized in the field is typically undiluted and therefore may result in even greater damage. Therefore, future work should seek alternative effective cleansers and/or methods which are approved for veterinary use which may have decreased effects to the dermal barrier and microbiota of the canine.

5. Conclusion

Repetitive bathing with highly dilute dish detergent to simulate decontamination practices resulted in significant impacts to the resident microbiota on canine skin. Of these changes is an increase in *Staphylococcus*, which has been previously associated with atopic dermatitis (2, 3, 5) and of which some strains may implicate human health (19, 20). Although the dish detergent utilized for the decontamination protocols was extremely dilute in comparisons to the typical undilute application commonly used in the field, the procedures affected some of the most prominent taxa of the canine dermal microbiota with no recovery to baseline abundance within the 35 days following the final bathing. Trends of these changes in alpha diversity metrics reached a low at day 7, which was only halfway through the bathing series, corresponding to half the time of standard SAR canine deployment. Should these practices be utilized in the field for the full deployment time of 14 days, it is expected that the canines will experience similar if not more severe disruptions to the dermal microbiota. It is additionally possible, that effects be seen after 7 days, however further work is needed to further explore this finding.

Moreover, these data reveal a heretofore unexplored relationship between changes in microbiota and coat condition and dander scores. These associations support that it is possible for changes in the dermal microbiota can be observed visually *via* changes in dander and coat condition assessments.

6. Study limitations

As the study was being conducted in Spring of 2020, the COVID 19 pandemic resulted in lack of access to a larger study population due to travel limitations for study technicians. Further limitations include lack of culture-based methods and lack of control population decontaminated with water alone. Future work should test for effects of water-only decontamination, utilize breeds commonly utilized in working disciplines including service and therapy dogs, and include microbial analysis from various anatomical regions to capture a more comprehensive picture of the entire dermal environment and impacts associated with bathing.

Data availability statement

The data presented in the study can be found in the online repository Figshare. Figshare doi: 10.6084/m9.figshare.23403308 can be found at https://figshare.com/projects/Impacts_to_canine_dermal_microbiota_associated_with_repeated_bathing/169451.

Ethics statement

The animal study was reviewed and approved by Southern Illinois University Institutional Animal Care and Use Committee (15–032) and the Royal Canin ethics committee.

Author contributions

DD participated in study design, data collection, data analysis, statistical analysis, and manuscript preparation. RK participated in study design, data collection, statistical analysis, and manuscript review. EP supervised all aspects. AW participated in study design and manuscript preparation and review. All authors contributed to the article and approved the submitted version.

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Conflict of interest

RK and AW were employed by Royal Canin SAS.

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

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2023.1204159/full#supplementary-material

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Assessing the impact of draught load pulling on welfare in equids

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About 112 million working equids are the source of income for 600 million people globally. Many equids are used for pulling loads (up to 15,000 kg per day) to transport goods. Most of them are associated with brick kilns, mining, and agriculture industries in developing countries. They may suffer from welfare issues such as overloading, being beaten, and being forced to work for long periods. These issues may occur due to a poor understanding of load-pulling equids. Understanding their capabilities and the elements that influence them is critical for efficient performance and welfare. The measurement of stride characteristics and gait kinematics can reveal loading adaptations and help identify loading limitations. It is known that both loading and fatigue change the locomotor patterns of load-pulling horses. Heart rate is a stress quantifying metric and an important representative of the speed of work and draught force. Heart rate variability is a regularly used statistic to quantify a physiological response to stresses, but it has never been used for load-pulling equids. Changes in blood lactate, nitrogen, oxygen, and carbon dioxide contents are reliable biochemical indicators of the effects of load pulling. Changes in plasma cortisol levels reflect the intensity of exercise and stress levels in horses while pulling a load. However, eye blink rate is a cheap, simple, and immediate indicator of acute equine stress, and we suggest it may be used to aid in load-pulling equine welfare assessment. However, further research is needed for a standardized and evidence-based draught load pulling capacity of working horses, mules, and donkeys.

KEYWORDS

donkey welfare, equine behavior, equine welfare, horse welfare, limb biomechanics, cart pulling, mule welfare, equine physiology

1. Introduction

The global equine population is approximately 116 million (1), and out of this, 112 million are working equids (2). Working equids are the source of income for their owners (3) and help to sustain 600 million people globally (4), most of whom live in poor and marginalized communities (5). Working horses, mules, and donkeys are vital to people's economic and social well-being (4). Carts hauled by horses, mules, and donkeys are essential modes of transportation in most of these communities, and carting is a source of income for a large proportion of the population in developing nations (Figure 1) (6). In low to middle-income countries (LMICs), while motorized transportation has grown quickly during the last few decades, the usage of equid's power to pull carts for local transportation of goods has remained unchanged (7). Carts are used to transport building materials, commercial produce, and garbage (2). Equids are crucial in the growth of agriculture and other activities as they provide power for plowing and traction, playing an important role in the local economy (8, 9). Equids' social and economic

FIGURE 1
Draught load pulling mule in a brick kiln production system (left) and fodder cart pulling donkey in a rural area (right) in Pakistan. Photo: Syed S. U. H. Bukhari.

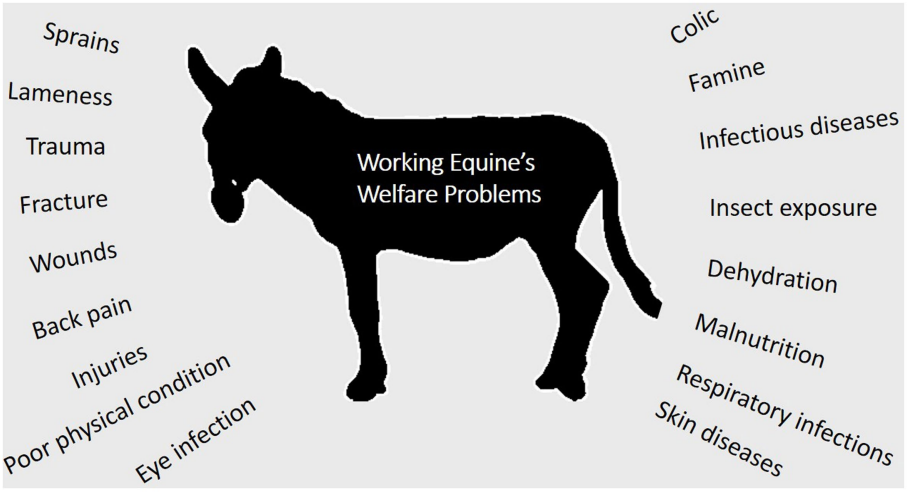

FIGURE 2
Common welfare problem of equids associated with draught load work.

contribution to rural earning can be direct (providing transportation services) or indirect (plowing the soil to obtain farm products) (10). Therefore, underestimating their contribution could have a negative impact on society (11), as they perform domestic tasks as well as agronomic and local transportation (12). Working equids are sometimes a person's only income source. They rely on them for day-to-day activities, providing access to medical care, access to schooling, and basic commodities to some of the globe's most marginalized communities (13, 14).

The welfare standards of working equids remain inadequate in LMICs (13, 15). Eye infections, infectious diseases, colic, skin diseases, poor physical condition, respiratory infections, back pain, injuries, exhaustion, wounds, malnutrition, famine, fracture, heat stress, dehydration (2, 10), trauma, insect exposure (16), sprains, lameness, as well as other catastrophic injuries (15, 17) are some of the most common welfare issues (Figure 2). They are prone to locomotor system diseases (18), which become even more common when subjected to hazardous working situations (19). Lesions caused by inadequate harnessing, dehydration, foot and shoeing issues, poor body condition score, and behavioral issues such as aggressiveness are the most common welfare issues observed in working equids (11). Donkeys often have a lower welfare standard than horses, with the most common issues being poor physical condition and injuries (3). This could be due to the fact that horses are more valuable and sell for a higher price. Over half of working equids endure starvation, fatigue, illness, and injuries during their working lifetimes, often exacerbated by a lack of accessible and cheap animal health treatments (20). Most equids have limited access to veterinary care, and most illnesses go untreated. The demise of an equid or even a reduction in the time it is able to work causes many difficulties for the community it serves (21). It is essential to ensure that each animal is pain-free, injury-free, and disease-free by providing prompt diagnosis and medical care (20).

Welfare problems of working equids are not limited to physical ailments; many working equids have behavioral issues, including fear of humans and sometimes even despair (22). Beating donkeys is one of the major causes of behavioral problems. Beating a donkey not only causes wounds and physical pain but it also induces fear and severe stress to the animal (23). Their poor welfare is connected to difficult operational conditions and handlers who lack basic knowledge of general husbandry and effective working equid care, such as

management of wounds, harness fitting and care, appropriate shelter arrangements, watering, veterinary services, and nutritional requirements (12). Donkeys and mules differ from horses in their behavior and require greater patience. If behavioral standards used to assess horses are used, their stoicism makes it more difficult to spot and diagnose problems in donkeys and mules (24). Working horses, mules, and donkeys, particularly in developing countries, must be considered in national livestock policy and programming (25).

The traction power of equids is used in brick-making industries in many LMICs (26). The work of horses, mules, and donkeys involves carting wet and dry bricks within brick kilns and from brick kilns to various places for use in the construction sector (10). In Egyptian brick kilns, donkeys are generally overloaded and may pull a cart averaging 2,040 kg in addition to the weight of the handlers, while suffering from pain and open lesions (12). In some LMICs, mules pull a draught load of about 1,500 kg per cart during a single trip, and there are about 8–10 trips per day (27), i.e., they pull about 15,000 kg per day. Moreover, equids making less frequent trips are 2.5 times more likely to carry heavier loads (28). Usually, a donkey (weighing 150–250 kg), working with brick kilns, transports 4,200 bricks (10,500 kg draught load) per day (29). Equines are frequently subjected to overwork and are regularly forced to work all day. Overworking is the cause of high prevalence of lameness in the young population of mules. This could be the reason of high turnover rate of working equids, with only 20% of animals owned for longer than 3 years (28). The most common concern is overloading, which exposes the animals to various wounds and back sores. The saddle and harnessing materials are frequently inappropriate, increasing the risk of equines suffering health and welfare issues (4). These issues may occur due to a poor understanding of the equid's needs or the owner's economic constraints.

Draught horses' physical work demands strength for pulling and endurance for prolonged labor. Endurance may be defined as the ability to perform a muscular activity at a high level of intensity for extended periods (30). It is important to understand the impact of draught load pulling on working animals, but many equestrian sports also rely on draught load. Harness racing is a prominent horse racing sport that evolved from a historic, recreational sport during which horses compete at a set gait while pulling a two-wheeled cart (31). Trotting and pacing are the two different gaits used in harnessed races. A trotter moves its legs forward in diagonal pairs (right front and left hind, then left front and right hind striking the ground simultaneously), whereas a pacer moves its legs laterally (right front and right hind together, then left front and left hind) (32). Horses were driven long before they were ridden, so driving is the oldest competitive equestrian sport. It is still alive and well in the twenty-first century. In competitive carriage driving, drivers sit in a vehicle drawn by a single horse, a pair of horses, or a team of four horses and compete in three events: dressage, marathon, and obstacle driving (33). Horses may also pull a heavy load in competition, for example, heavy horse pull competition at the Calgary Stampede (33, 34). These sports have significant economic benefits for society (34).

Load pulling equids are of great value as they are used both as working equids in LMICs and in harness competitions internationally. However, most research focuses on ridden horses. People who use horse power should be aware of their limitations to maximize equine welfare. Understanding equines' labor capacities including their load pulling abilities (how much they can/should pull?), which might influence their optimum field performance, is critical to their efficient utilization. Quantified load pulling limits could then be used by non-governmental organizations (NGOs), policymakers, and other stakeholders working with vulnerable communities and working equids to limit excessive load pulling and improve animal welfare. The biomechanical, physiological, biochemical, and behavioral impacts of pulling load on equids are discussed in this review.

2. Biomechanical assessment

To understand the biomechanical effects of load pulling on equines, a basic understanding of the mechanics of load pulling is needed. Draught force can be defined as the force required to pull a load in the same direction of travel as the horse (30, 35). Horses pulling loads experience different forces. With a draught angle of zero and shafts parallel to the ground, the horse only needs to exert a horizontal force to move the load (Figure 3) (36). When there is a draught angle, the shafts are at an angle to the ground, and the horse must exert the same horizontal force and a vertical force because the load is pulling back and down on the horse (Figure 4) (36). This is related to the observation that horses can move faster, pulling rather than carrying a given load at a given gait (17). Therefore, horses dragging loads are exposed to different forces and are prone to a different set of injuries than horses carrying loads (36). The biomechanics of equine load pulling is not well studied. At the start of work, when horses are initially loaded by the horizontal-pulling load, their general movement pattern remains unchanged. More drastic variations in the movement pattern occur due to fatigue (37).

The measurement of stride characteristics and gait kinematics can reveal loading adaptations and help identify loading limitations (17). It is known that both loading and fatigue can change a horse's locomotor pattern (37). In general, changes to stride patterns with speed are conserved across breeds; both Thoroughbred and draught horses tend to increase speed by increasing stride length more than stride frequency (38). When pulling a draught load, however, an increase in stride frequency and a decrease in stance time are seen (37). Stride frequency increases from 108.2, 105.4, and 108 strides/min to 117.2, 118.8, and 119.6 at the same speed of 9 ms^{-1} with 0.1kN, 0.2kN, and 0.3kN draught force, respectively (39). At similar canter speed (8 ms^{-1}), stride frequency is greater in Thoroughbred than in draught horses, as mean stride frequency remains at 110.4 and 100.8 strides/min, respectively (38). Interestingly, during incremental (0.2 kN increased every 2 min) draught force exercises starting from 0.04 kN, stride length remained constant and did not change (40). In contrast to this, in another investigation, horses did reduce their stride length (from 3.74 to 3.65 m) in response to increased pulling load (0–34 kg) (37). Furthermore, stride length increases from 3.1, 3.5, and 3.1 m to 4.1, 4.6, and 4.1 m with a draught force of 0.1kN, 0.2kN to 0.3kN at the same speed of 9 ms^{-1} in load pulling horses, respectively (39). However, while slow trotting (3 ms^{-1}), stride length is determined by the speed regardless of increasing weight resistance (40).

Comparing Thoroughbred and draught horses, the walk to trot transition is about two ms^{-1} in both breeds (38). The transition from trot to canter is between 4–6 ms^{-1} for Thoroughbred and 6–8 ms^{-1} in draught horses with draught force of 0, 5, 10, 15, and 20% of their body weight, respectively. However, changing the draught force does not affect gait type at any speed (38). As at a given pace, higher draught

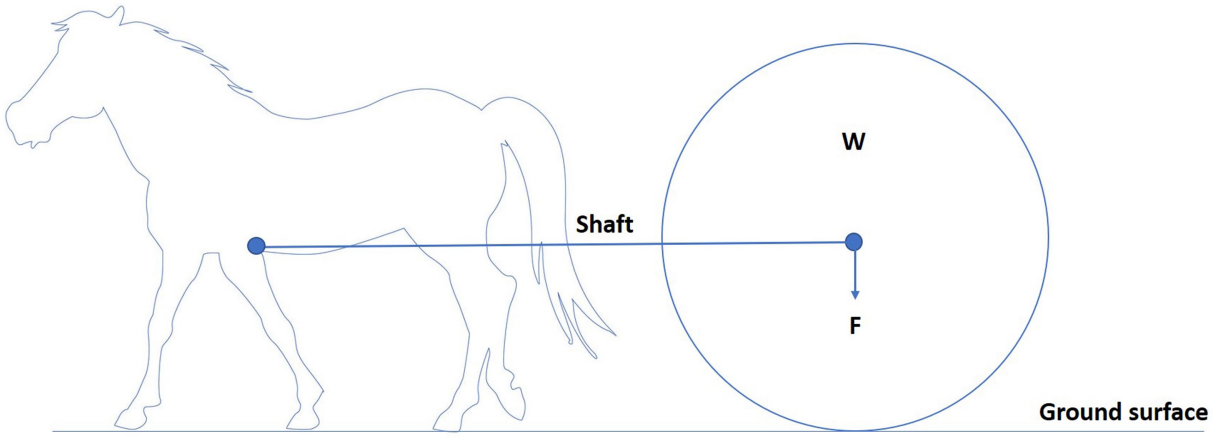

FIGURE 3
Horse pulling a load (W) with a zero-draught angle as the shafts are parallel to the ground. The arrow indicates the direction of the load's force (F). The horse only needs to exert a horizontal force to move the load.

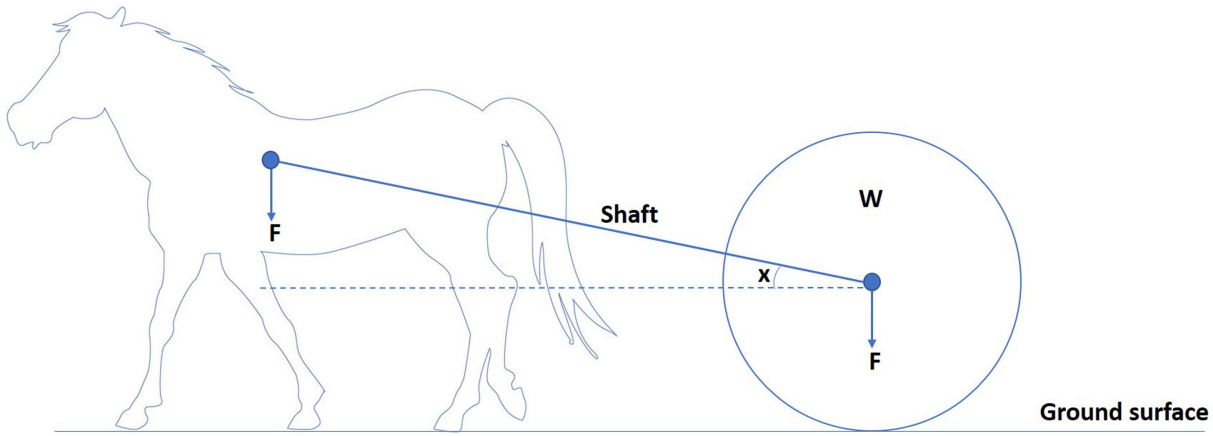

FIGURE 4
Horse pulling a load (W) with a draught angle (x) as shafts is not parallel to the ground. The arrow indicates the direction of the load's force (F). The horse needs to exert both horizontal and vertical force to move the load, as the load is pulling back and down on the horse.

force is associated with increased stride frequency and shorter stride length. When we increase speed without a draught force and change force at a constant pace, the stride characteristics of Thoroughbred and draught horses are comparable (38).

The period of time when the foot is in contact with the ground's surface is known as stance time (17). When pulling a load, horses reduce stance time in both forelimb and hindlimb (37). Horses reduce their stance time (from 0.165 to 0.157 s) in response to an increase in pulling load (from 0 to 34 kg), in contrast to horses under mounted, load, which increase their stance duration (17, 41). This disparity between load pulling and mounted load could be attributed to differences in vertical ground reaction forces or limb load, but this has not been well investigated in horses, mules, and donkeys. This could be because, theoretically, a draught angle of zero (shafts parallel to the ground) requires the horse to exert just a horizontal force to move the load (36); therefore, the load should not cause an increase in vertical ground reaction force on the limbs.

A walking horse (weighing 648 kg) with a speed of 2.11 ms^{-1}, pulling 1,892 kg load for 4 h, generates a draught force of 0.59kN and produces a work of 15.69 MJ (42). Horses work more quickly on the first day of their work after some rest; during later days, they become slower. Compared to buffaloes or oxen hauling carts over a flat surface, a horse's average pace is twice as fast (43, 44). Horses weighing 675–860 kg can constantly work at a rate of 0.75 kW for 10 h a day and travel a total of 32.2 km per day without becoming fatigued (42). While measuring time-averaged draught force (TADF) and distance-averaged draught force (DADF), it was observed that the differences between TADF and DADF can be minimal for low loads pulled by large and well-trained oxen. In contrast, time averages can offer bigger and unpredictable values for large jerky loads pulled by small and inexperienced animals (45). This could be because animals slow down when confronted with a large draught force.

Interestingly, donkeys can cover a distance of 20.5 km while working continuously until exhaustion with a speed chosen by

themselves and pulling a load equivalent to 21% of their body weight (46). When the draught load increases from 500 to 600 and 700 kg in working donkeys weighing 159 kg, the work speed begins to decline from 0.97 to 0.81 and 0.70 ms^{-1}, respectively, suggesting that speed and applied loads are inversely related (47). Speed is an essential parameter for assessing donkeys' limits of pulling a load, as a voluntary decrease in speed appears to be a reliable predictor of fatigue in donkeys (46). Therefore, donkey owners and working equine welfare advocates can use this indication to determine donkey loading limitations. Though donkeys are generally referred to as "pack animals," research has shown that they are highly efficient at pulling loads. Donkeys can pull about 2.7 times of their live weight. However, suppose the donkey is subjected to continuous and long working hours (almost 6 h). In that case, it is recommended to keep the load about double of their live weight to safeguard the donkey's welfare (47).

Donkeys are more efficient in carrying and pulling loads than oxen and buffaloes. The energy costs of pulling loads (5–18 kg) by donkeys are 26.5, 15.3, and 6.2 $J\,m^{-1}\,kg^{-1}$ at 0, −10%, and − 15% slope (48). The energy cost is lower at a higher downward slope, as donkeys may be fully utilizing the potential energy of their body weight and the load, probably reducing the energy cost of locomotion. However, as the work rate increases, the efficiency of performing work decreases in donkeys. Their response to exercise is strikingly similar to that of the horse in several parameters, including the extent of its aerobic capacity and locomotor efficiency (49). However, due to the adaptations of the Thoroughbred and Standardbred for high-intensity work, research involving non-racing breeds of horse may be more relevant for studying and predicting donkey performance. For example, it is important to note that there is a difference between walking patterns of donkeys and horses, with some evidence that they walk with a lateralized stride pattern nearing a pace (50) rather than a true four-time walk which is usually observed in horses (51). The donkeys had a shorter stride time (0.87 s), stance time and swing time (forelimbs only) in comparison to previous studies in ponies walking with same speed (1.25 ms^{-1}), but have similar swing phases in the hindlimbs (50, 51). This could indicate that the biomechanical consequences of loading investigated in horses cannot be simply translated to donkeys. It is also recognized that donkeys and horses differ physiologically, so results from horse research may not be applicable to donkeys (7, 17, 52).

Standardbred racehorses pulling a small carriage (a "sulky") suffer different injuries to Thoroughbred racehorses racing with a rider. Musculoskeletal injuries are the leading cause of reduced training days and racehorse wastage (31), and so injuries in Standardbred and Thoroughbred racehorses and the differences between them are well-studied. Many researchers have concentrated on their unique concerns, such as injuries to the middle carpal joint (53) and fractures of proximal sesamoid bone (54). This may be due to uncommon catastrophic accidents during competitions, therefore there are fewer concerns about the safety of races associated with load pulling (31). For example, tibial stress fractures are rare in load pulling racehorses, as are lateral condylar fractures and biaxial proximal sesamoid bone fractures (31). Improved gait mechanics and efficiency can be achieved with age and training in load-pulling racehorses (55). The lack of catastrophic injuries such as suspensory breakdown in load pulling racing may be related to slower speeds and a more caudal position of the center of mass compared to Thoroughbred racehorses (56, 57). Age, gender, driver, racing speed, racing intensity, racing shod, and medical treatment are potential risk factors concerning musculoskeletal injuries (31). However, Injuries in working horses and donkeys pulling loads are less well studied.

In addition to the impact of loading on gait biomechanics, it is important to consider the effects of fatigue. Fatigue increases injury risk (37), and is likely to impact on the welfare of working equids pulling loads for long hours. Generally, increased stride length and stance time are seen in horses due to locomotor fatigue. Johnston et al. (37) tested Standardbred horses, fatigue increases stride length (from 3.74 to 3.87 m) in response to a pulling load of 34 kg, working with a speed of 7 ms^{-1}, and stance time reverts back to a non-loaded value (from 0.157 to 0.165 s) (37). Swing time does not change with loading, but does alter with fatigue, increasing from 0.370 to 0.394 s (37). Finally, as a result of increased joint excursion during the stance phase, the forelimb and hindlimb become more flexed due to fatigue (36). Heavier loading may cause a shorter vertical displacement and a stronger forward impulse from hindlimbs to the horse's body (36, 37).

3. Physiological effects of loading

Physiological indicators such as blood temperature (58, 59), rectal temperature (39, 42, 46, 60, 61), heart rate (30, 39, 40, 42, 46, 47, 49, 61, 62), respiration rate (30, 39, 42, 46, 61), hematological profile (30, 40, 49, 61, 62), muscle fiber composition (39, 40, 63), creatinine kinase (62, 64, 65), lactate dehydrogenase (42, 62, 63), alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, citrate synthase, and 3-hydroxy acyl-CoA dehydrogenase (42, 63, 64), have been investigated in relation to the load pulling capabilities of equids (Figure 5). However, the conditions under which this work has been done have been highly variable, so generalization of the results is difficult. Moreover, there is no research available on working equids in field conditions in LMICs, which often have high temperatures, high humidity, and rough terrain. The physiological impact of load pulling in field conditions would be different from ideal indoor conditions.

Additionally, donkeys are frequently utilized for load pulling, despite the fact that the majority of study on the impact of pulling load has been undertaken on horses. This is significant because donkey physiology frequently differs from horse physiology (7, 52). Compared to horses, donkeys have a lower resting body temperature (36.5–37.7°C), higher resting heart rate (31–53 beats/min.), and higher respiration rate (13–31 breaths per min) ranges (17, 52). Donkeys have fewer erythrocytes (i.e., a lower packed cell volume), but they are larger than those in horses (7). Therefore, there is a need of detailed research on the impact of load pulling on donkey's physiology.

Compared to many other species, the horse has an obvious disadvantage for heat dissipation as it has a high metabolic capacity, but a small surface area, especially since sweat evaporation is the primary method of heat dissipation (66). During work, temperature increase is more rapid with 1 min of exercise at VO2max as compared to 62% VO2max (being 38.3°C and 37.9°C, respectively). However, blood temperature at fatigue remains the same for both VO2max and 62% VO2max, that is, 41°C (59). This is important because if an animal is fatigued while pulling a load, the work intensity does not matter in relation to metabolic heat production. In trotting horses (9 ms^{-1}), rectal temperature increases from 37.9°C to 39.2°C irrespective to level of draught forces (0.1, 0.2, and 0.3 kN) (39).

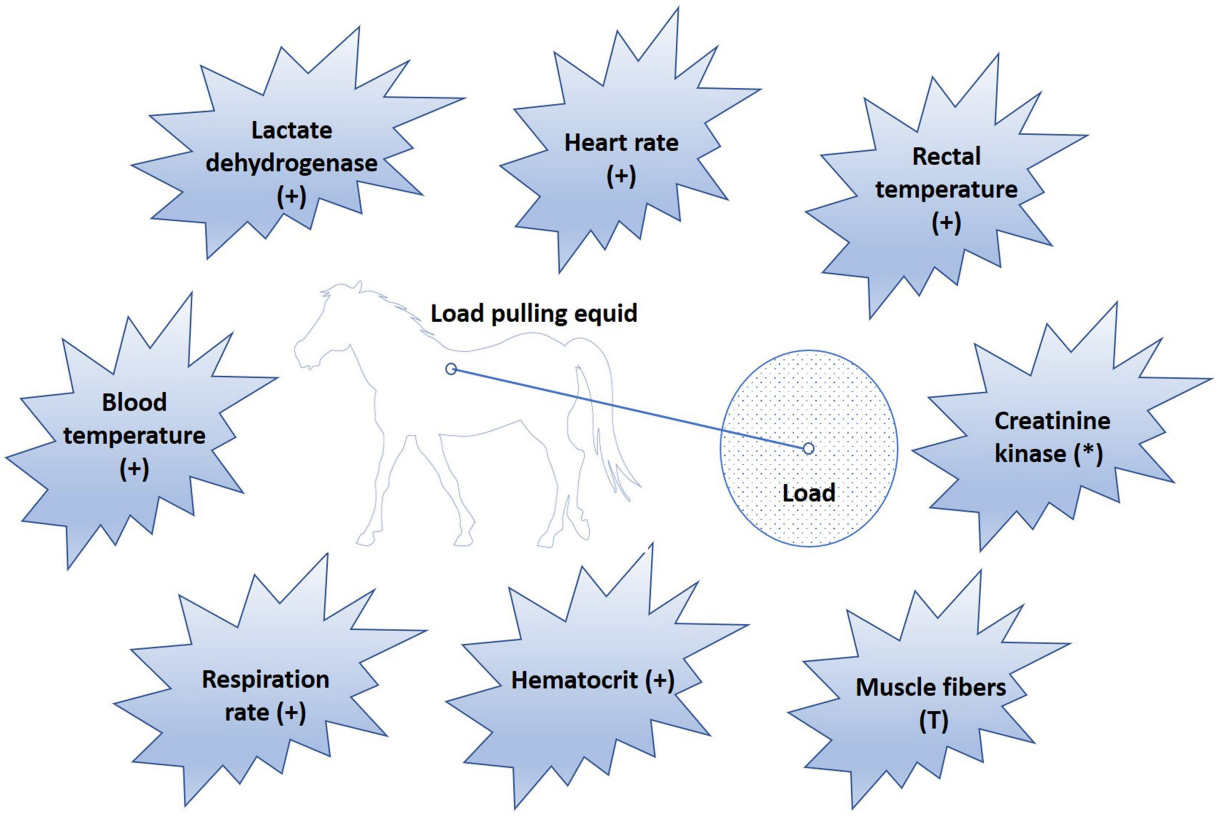

FIGURE 5
Physiological parameters studied in relation to load pulling in equids. Sign (+) indicates, value of the respective parameter increases in response to work. Symbol (*) indicates, parameter value increases in untrained equids but remains normal in trained working animals. Sign (T) indicates that as work intensity increases, muscle fibers are recruited in the order from type I to IIA, and IIB.

Interestingly, horses working with a speed of 2 ms^{-1}, an increase in draught force from 0.33 kN to 0.78 kN do not result in a significant increase in rectal temperature (38.3°C–38.5°C) (60). However, in competition horses, pulling 2.5 times their body weight over a 60-m hard beach sand track for 1.2 min, rectal temperature increases from 37.8°C to 38.4°C (61). In horses (weighing 648 kg) working continuously for a longer period (4 h), rectal temperature increases (from 37.7°C to 38.5°C) with exercise consisting of 0.59 kN draught force over a distance of 26.63 km with a speed of 2.11 ms^{-1}. However, this change in rectal temperature recovers within 2 h of rest after the end of exercise (42). In donkeys, pulling load equivalent to 21% of their body weight (235 kg), trotting with a speed of 2.5 ms^{-1} for 30 min, rectal temperature increases from 37.2°C to 39.3°C. However, this change in rectal temperature does not recover even after 1 h of rest after the end of exercise (46). This may be due to the faster metabolic rate in donkeys than horses (64), but this has yet to be investigated in relation to load pulling. These studies indicate that work speed may be more important than draught force in influencing body temperature, but a direct comparison between work speed and draught force in load pulling equids is never made.

Heart rate is a stress quantifying metric used to define stress levels under continuous work. It is known that heart rate is a vital parameter for instant evaluation of health status, training load, and adaptability of equids (7, 31). Workload is reflected in the heart rate reaction to exercise, which increases linearly with the larger the draught load (37, 39, 40, 49). Heart rate increases (from 41 to 76 bpm) with exercise consisting of 0.59 kN draught force over a distance of 26.63 km with a speed of 2.11 ms^{-1} in horses of 648 kg body weight. This change in heart rate does not recover even after 2 h of rest after exercise (42). However, in competition horses, pulling 2.5 times their body weight over a 60-m hard beach sand track for 1.2 min, heart rate increases from 40 to 105 bpm (61). Therefore, the working terrain friction coefficient (FC) is critical for calculating equid load pulling capacity (67). Heart rate is also an important indicator of the speed of work in addition to draught force (40). While explaining the impact of load pulling on working equids, work speed may have greater importance than actual draught force. As the working speed of horses increases from 6 ms^{-1} to 9 ms^{-1} with a constant draught force (0.2 kN), heart rate increases linearly from 167 to 203 bpm (39). Therefore, it is essential to consider both pulled weight and speed of working for accurate quantification of load pulling abilities of equids.

Respiration rate is a function of speed and draught force similar to heart rate, i.e., respiration rate increases with the increase in draught load, but this change in respiration rate is less when compared with the effects of mounted load on respiratory responses (68). Work consisting of 0.59 kN draught force over a distance of 26.63 km at a speed of 2.11 ms-1, respiration rate in horses (weighing 648 kg) increases (from 24 to 52 breath/min). This change in respiration rate does not recover even after 2 h of rest after exercise (42). This could be due to the animal being severely overheated, as respiration should return to normal if the horse is used to working. However, in competition horses, pulling 2.5 times

their body weight over a 60-m hard beach sand track for 1.2 min, respiration rate increases from 32 to 56 breaths/min (61). Hence, the working terrain (FC) is important for calculating equids' load-hauling capabilities (67). In trotting horses (9 ms^{-1}), a greater increase in respiration rate, from normal to 110, 119, and 104 breath/min, has been seen with a less draught force of 0.1, 0.2, 0.3 kN (39). Even though the draught force is low, this increase in respiration rate is caused by work speed. As the working speed of horses increases from 6 ms^{-1} to 9 ms^{-1} with a constant draught force (0.2 kN), the respiration rate increases from 94 to 119 breath/min (39). Therefore, for accurate estimation of load pulling capacities of equids, it is necessary to account for both hauled weight and working speed.

The hematological profile is essential in determining physiological changes occurring in equids (65). Hematological parameters change due to exercise in both horses and donkeys (17). Both draught weight and work speed are proportional to changes in red cell volume. The red cell volume is thought to measure the horse's oxygen transport capacity (40, 69). Furthermore, the horses hauling the heaviest weights and trotting at the maximum trotting speeds have the highest red cell volume (40). In a team of two horses (one colt and one stallion), pulling draught load of 0.93 kN draught force while working together for 150 min, hematocrit (Hct%) increases from 34.8 to 42.6% and from 37.8 to 45.5% in colt and stallion, respectively (62). In donkeys working with a speed of 1.8 ms^{-1} and a draught force of 0.4 kN for 25 min, Hct% increases from 39 to 48.6% (49). This implies that aerobic capacity is necessary for both draught-loaded exercise and work speed, both of which are crucial factors for quantifying the impact of load hauling on equids.

Horses are believed to get stronger, increase muscle volume, and have enhanced endurance due to load-related workouts (39). The need for force grows as the draught load increases, and the rate of energy expenditure in the muscles may surpass the horse's maximum rate of oxygen supply, so the oxidative capacity of the muscles is important (40). In muscles, type I fibers have a low ATPase activity, and a high oxidative capacity, and a low glycolytic capacity. Type IIA fibers have a high myosin ATPase activity, and a high oxidative and glycolytic capacity. Type IIB fibers have a high myosin ATPase activity, and a low oxidative capacity, and a high glycolytic capacity (70). As work intensity increases, fibers are recruited in order, from type I to IIA to IIB. Type I and a significant proportion of type II fibers are recruited at rapid trotting speeds (40). Type I and IIA muscle fibers (in the gluteus medius, longissimus, and brachiocephalicus muscles) increase, while type IIB muscle fibers decrease in response to a 12-week draught loaded exercise test (0.33 kN draught force, with speed ranging from 5.5 to 8 ms^{-1} for 12 min) (63). Compared to draught horses, Thoroughbreds can exert the same draught forces and reach double the speed, external power, and oxygen consumption. Thoroughbred horses' maximum oxygen consumption is reported to be roughly twice that of draught horses, showing adaptations to high-intensity activity (38). Compared to Thoroughbred horses, draught horses' peak efficiency occurs at lower speeds, demonstrating adaptations to high-force and low-speed activities. The disparities in force, oxygen consumption, and peak efficiency speed between draught horses and Thoroughbreds are most likely due to distinct locomotor muscle contraction velocities (38), and maybe due different muscle fiber types (type I, type IIA, and IIB). These disparities in locomotor muscle contraction velocities, and the order in which muscle fiber types are recruited, have yet to be explored in donkeys and mules.

Creatinine kinase is a muscle-specific enzyme with a half-life of 2 h in the blood (42). In horses, a spike in serum creatine kinase enzyme activity is a helpful diagnostic of post-exercise muscle soreness and muscle injury (17). Since its rise in plasma activity is greater in untrained horses than in trained horses, measuring creatinine kinase concentrations could be a helpful fitness indicator (42). At a maximum load that a horse can pull over a distance of 14 feet, during heavy horse pull competition at Calgary Stampede (71), creatine kinase enzyme activity increases from 174 to 225.5 IU/L (64). However, in a team of two horses (one colt and one stallion), pulling draught load of 0.93 kN draught force while working together for 150 min, creatine kinase enzyme activity increases from 62.1 to 101 U/L and from 127 to 167 U/L in colt and stallion, respectively (62). On the second day of work, it may recover to its baseline levels (42), after which the values can remain within the normal range (for colts, 62 ± 52 U/L; for stallions, 127 ± 67 U/L) (62). This would show that the equids have adapted to load-pulling work. Hence, changes in creatinine kinase activity in the blood may be a reliable indication of an equids' aptitude for load-pulling work.

The lactate dehydrogenase enzyme is commonly found in muscles (63). Although it is usually believed that an increase in the concentration of muscle enzymes in plasma indicates muscle damage, given the slight variations in these enzymes' values within normal ranges described for horses, it is possible that the changes in these enzymes' values are due to changes in the permeability of the muscular cell membrane (42). In a team of two horses (one colt and one stallion), pulling draught load of 0.93 kN draught force while working together for 150 min, lactate dehydrogenase activity in blood increases from 634 to 785 U/L and from 604 to 646 U/L in colt and stallion, respectively (62). No change in lactate dehydrogenase activity occurs (in the gluteus medius, longissimus, and brachiocephalicus muscles) in response to a 12-week draught loaded exercise test (0.33 kN draught force, with speed ranging from 5.5 to 8 ms^{-1} for 12 min) (63). However, the length and intensity of exercise positively correlate with the rise in plasma enzyme activity after exercise. This rise can be mitigated with proper training (62).

4. Changes in biochemical indicators

Biochemical indicators such as blood lactate (38, 39, 49, 62, 72, 73), blood oxygen, blood carbon dioxide level (42, 59), blood glucose (42, 60, 62, 64, 73), and adenosine tri phosphate has been investigated in relation to load pulling (59, 60). Moreover, sodium, chloride, potassium (61, 64), plasma protein (61, 64, 74), uric acid, urea (61, 62, 64, 73), plasma triacylglycerols, free fatty acids, and cholesterol (60, 62, 73) have also been investigated in relation to load pulling capabilities of equids. However, these studies have used different parameters in different conditions and the number of studies is insufficient for each parameter to provide a comprehensive understanding of the effect of draught load on the biochemical parameters and quantification of draught load pulling abilities of equids. Therefore, it may be important to quantify their load pulling ability in standardized working conditions.

The lactate concentration in the blood is a reliable indicator of the load effect (17) because the commencement of anaerobic metabolism

is signaled by increased blood lactate levels, which is related to a reduced ability to maintain a given exercise level in equids (49). In response to load pulling, blood lactate levels rise sharply (40), and it increases exponentially with an increase in draught force and velocity (40, 68). In working horses (9 ms^{-1}), as the draught force increases from 0.1kN to 0.3kN, plasma lactate rises from 3.8 to 10.8 mmoL/L. Similarly, with the increase in work speed from 6 ms^{-1} to 9 ms^{-1}, with a constant draught force (0.3 kN), plasma lactate increases from 4.5 to 10.8 mmoL/L (39). If we compare Thoroughbred and draught horses, plasma lactate increases from resting level (0.8 mmoL/L) to 7.3, 12.4, 11.4, 10.5, 6.7 mmoL/L and from resting level (0.8 mmoL/L) to 4.4, 12, 12.6, 7.3, 12.7 mmoL/L in Thoroughbred and draught horses, with draught force equals to 0, 5, 10, 15, and 20% of their body weight, respectively (38). This demonstrates the metabolic difference between Thoroughbred and draught horses at lower and higher levels of load pulling, although there is no doubt that both breeds use anaerobic metabolism at various levels. Furthermore, when comparing a young and experienced horse, the older horse has a lesser increase in blood lactate as the adaptation to pulling load occupation develops with the passage of time (62).

In horses, skeletal and cardiac muscle oxygen requirements rise in proportion to their metabolic needs. The main limiting elements in intensive muscular exertion are oxygen-carrying functions of the circulatory system and oxygen use in muscles (30). In horses (weighing 648 kg) working continuously for a longer period (4 h), arterial oxygen level (pO_2) decreases from 103 mmHg to 93.8 mmHg, and venous pO_2 increases from 46.8 mmHg to 51 mmHg. Whereas, arterial carbon dioxide level (pCO_2) increases from 32.9 mmHg to 35.4 mmHg, and venous pCO_2 decreases from 36.5 mmHg to 35.3 mmHg with exercise comprising of 0.59 kN draught force over a distance of 26.63 km with a speed of 2.11 ms^{-1} (42). The oxygenation of arterial blood during exercise decreased, limiting oxidative metabolism (59). Although an increase in venous pO_2 appears to reflect a decrease in tissue oxygen consumption, it could just be a redirection of blood flow to places like the skin to aid heat dissipation. As they took jugular blood samples for venous pO_2 (30), which is venous drainage from the head and neck areas where oxygen use may be reduced during exercise, causing an increase in venous pO_2 during work.

The use of glucose in the muscle during load-pulling exercises is determined by the weight of draught load and the duration and speed of work (42). The most common reaction of horses to pulling load at low speeds for long periods is either no change or reduced blood glucose concentrations (42). In horses working with a speed of 2 ms^{-1} and a draught force of 0.33 kN, blood glucose level decreases from 5.6 to 4.4 mmoL/L (60). Interestingly, at a maximum load that a horse can pull over a distance of 14 feet, blood glucose level remains unchanged during heavy horse pull competition at Calgary Stampede (64, 71). However, in mules (320–380 kg bodyweight), working under a draught load equals 10% of their body weight for 2 h, blood glucose level decreases from 5.417 to 4.917 mmoL/L (73).

Adenosine triphosphate (ATP) is also affected by load-pulling inside horses (59). Interestingly, no marked changes occurred in the levels of muscle ATP in horses working with a speed of 2 ms^{-1} with either a draught force of 0.33 kN or 0.78 kN (60). In an identical fashion, no marked changes occur in the level of muscle ATP in response to exercise at 62% of VO2max. However, ATP contents decrease significantly in response to exercise at VO2max (59). Intense exercise, demanding more oxygen and energy, can reduce ATP level, and it is not affected by less energy-demanding work.

Fluid and electrolyte losses can compromise optimum exercise performance (75, 76). At a maximum load that a horse can pull over a distance of 14 feet, during heavy horse pull competition at Calgary Stampede (71), plasma sodium, chloride, and potassium decreases from 129.5 to 125.5, 95 to 92, and 3.3 to 2.9 mmoL/L, respectively (64). In contrast, in another study, pulling exercise caused a short-term elevation in sodium and chloride, which rapidly returned to resting values within 15 min in horses (61). During exercise and recovery, the renin-angiotensin-aldosterone axis (RAA) is linked to the acute and chronic defense of blood pressure, plasma volume, along with fluid and electrolyte balance (74–76). Furthermore, acute hypovolemic stress activates the RAA axis (74), and high aldosterone and arginine vasopressin concentrations are associated with exercise in horses (75, 76). Exercise has little effect on renin levels, although it does increase aldosterone and arginine vasopressin levels (74).

In horses, plasma protein contents are affected by load pulling work (61), but it is likely to be due to dehydration level, not due to duration or intensity of work. In horses, pulling 2.5 times their body weight over a 60-meter hard beach sand track for 1.2 min, total plasma protein increases from 7.8 g/dL to 8.5 g/dL, and plasma albumin increases from 3.5 g/dL to 4 g/dL (61). Interestingly, at a maximum load that a horse can pull over a distance of 14 feet, during heavy horse pull competition at Calgary Stampede (71), total plasma protein, albumin, and globulin remained the same before and after the competition (64). However, the level of total plasma protein and albumin critically depends on the hydration status of horses (74, 77). If the horse is dehydrated, he will have a higher level of total plasma protein contents per unit volume of plasma.

Generally, blood nitrogen contents (uric acid and urea) increase after load-associated work in equids (17, 61, 62, 73). In a team of two horses (one colt and one stallion), pulling draught load of 0.93 kN draught force while working together for 150 min, plasma uric acid increases from 0.014 to 0.041 and from 0.017 to 0.026 mmoL/L in colt and stallion, respectively (62). As far as plasma urea level is concerned, in horses, pulling 2.5 times their body weight over a 60-meter hard beach sand track for 1.2 min, total plasma urea contents increase from 7.2 mmoL/L to 9.5 mmoL/L (61). However, at a maximum load that a horse can pull over a distance of 14 feet, during heavy horse pull competition at Calgary Stampede (71), plasma urea contents remained the same before and after the competition (64). Interestingly, in mules (320-380 kg bodyweight), working under a draught load equals 10% of their body weight for 2 h, serum urea increases from 8.7 to 12.8 mmoL/L (73). Plasma nitrogen concentration is considered a parameter of overtraining in humans (78). Therefore, a rise in plasma nitrogen contents could be a concern and an important indicator of load pulling limits in equids.

Plasma triacylglycerols and free fatty acids (FFA) are crucial biochemical measures to understand the impact of pulling a load in equids because the changes in plasma triacylglycerol levels reflect the intensity of exercise (62, 79), and plasma FFA represents important oxidative metabolic substrates, especially when pulling load for long periods. In a team of two horses (one colt and one stallion), pulling draught load of 0.93 kN draught force while working together for 150 min, plasma triacylglycerol increases from 0.28 to 0.66 mmoL/L and from 0.31 to 0.53 mmoL/L in colt and stallion, respectively (62). This increase of triacylglycerol is specific for exercising horses; it was

not observed in rodents or human beings. In horses working with a speed of 2 ms^{-1} and a draught force of 0.33 kN, plasma free fatty acids (FFA) increase from 300 to 790 µmoL/L. During post-exercise resting intervals, FFA levels increased more than during walking intervals (60). It is important to remember that, during load pulling work, horse FFA usage varies depending on draught resistance, velocity, and duration of activity (60). Furthermore, In mules (320-380 kg bodyweight), working under a draught load equals 10% of their body weight for 2 h, blood cholesterol level decreases from 2.570 to 2.239 mmoL/L (73), which may be due to their utilization during load pulling work. However, these studies were performed under different conditions; a standardized approach may be used to compare these parameters better and understand the impact of pulling load on equids.

5. Behavioral measures and indicators of stress

The use of behavioral cues to evaluate the impact of draught load in equids is still in its early stages. Assessment of donkeys' stress responses are always conducted based on irregular behavioral phenomena that may be difficult to interpret (80, 81). Behavioral responses are the first line of defense to environmental challenges and stress. In donkeys, signs of fatigue include unwillingness to continue, uncoordinated legs and excitement after work (47). Behavioral problems like hyperesthesia, depression, non-responsiveness, avoidance, aggressive response, and avoiding chin contact have been observed in donkeys pulling heavy brick kiln load (23, 82). An improved general attitude and reaction to observers are associated with an improved body condition. As a consequence, it is important for the owners of working donkeys to pay attention to changes in their body condition in order to avoid compromising their welfare (12). Draught load associated changes in the donkey's behavior are shown in Figure 6. While ridden changes in behavior due to loading have been investigated in horses (83), draught load associated changes in horse and mule behavior have yet to be investigated.

The speed of draught load pulling donkeys is also an important behavioral measure, as it has an inverse relation with the weight of load (47). Therefore, speed is an important parameter for assessing donkeys' limits of pulling a load, as a voluntary decrease in speed appears to be a reliable predictor of fatigue in donkeys (46). Working donkey owners should pay attention to the speed of walking donkeys and take necessary measures to avoid compromising donkey welfare and performance during their routine work.

Equine stress must be measured to assess an equid's emotional state and welfare. An ethogram has been used to assess musculoskeletal pain-associated behaviors in horses (84), which may only be helpful when used by trained assessors (85). More recently, a grimace-scale for assessing pain has been developed for use in donkeys (86), although this has not yet been used in the field with working donkeys. While no ethogram has been designed for load-pulling horses, mules, and donkeys, other measures that are easy to assess in the field are becoming available. Recently it has been reported that eye blink rate is a cheap, simple, and immediate indicator of acute equine stress (87–89). As it has been seen that in the presence of a stressor (presentation of the clipper), blink rate first decreases (7 blinks/min) and then go higher (13 blinks/min) than the resting blink rate (10 blinks per min) in stable horses (87). Therefore, it may aid in pulling load equine welfare assessment (87–89). Traditional stress measurement techniques, such as heart rate, heart rate variability (HRV), cortisol level, and more recently, changes in eye temperature (17, 90, 91), need special equipment which are not readily available in the equine's working environment. However, the use of

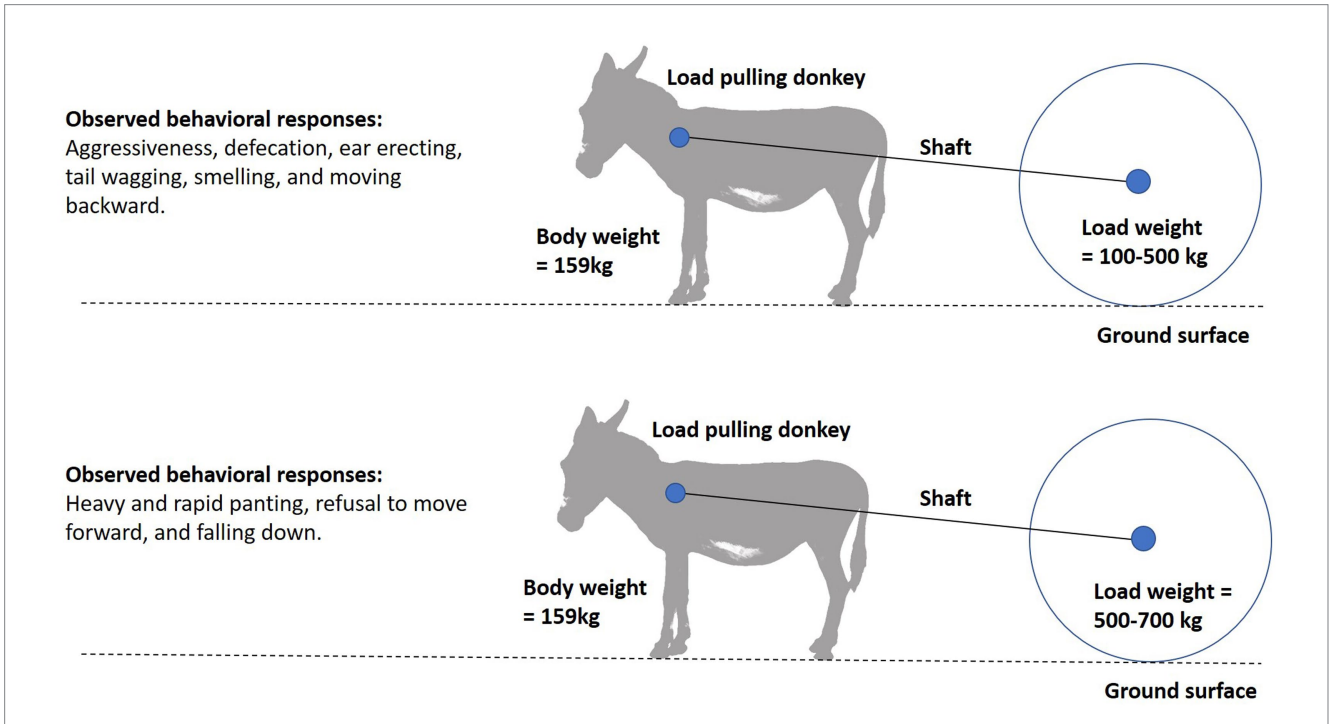

FIGURE 6
Behavioral responses of load pulling donkeys in response to different load weights (47).

spontaneous blink rate for stress assessment needs to be investigated in working equids.

In animal science, heart rate variability is a regularly used statistic to quantify a physiological response to stresses. HRV analysis relies on accurate detection of the heart's electrical activity (90). Heart rate variability is the variation in the time interval between heartbeats. It decreases with heavy riders (20% body weight ratio) as compared to lighter riders (10% body weight ratio) (92). There is no study available assessing HRV association with pulling load for working horses, mules, and donkeys.

Cortisol is not a good measure of work and load-related stress because it may also be significantly affected by diet, genetic factors, environment, and characteristics associated with individuals (93, 94). Generally, changes in plasma cortisol levels reflect the intensity of exercise (62), stress level, including exercise-induced stress in equids (49). In a team of two horses (one colt and one stallion), pulling draught load of 0.93 kN draught force while working together for 150 min, plasma cortisol increases from 382.5 to 785 and from 234.7 to 482.5 nmoL/L in colt and stallion, respectively (62). Moreover, in donkeys (weighing 183 kg), plasma cortisol increases from 76 to 399 nmoL/L with a draught force of 0.4 kN for 25 min with a speed of 1.8 ms^{-1} (49). However, salivary cortisol measurement is far superior to plasma cortisol measurement for assessing stress and hypothalamus-pituitary–adrenal activity because it avoids the need to account for between-subject differences in cortisol binding globulin or within-subject alterations (17). Here, the difference in cortisol levels between horses and donkeys could be due to the difference in duration and intensity of exercise. Moreover, it is known that donkeys' response is similar to horses as far as plasma cortisol level is concerned (49).

6. Conclusion

One of the many issues that may jeopardize working equine welfare is pulling overly heavy loads. Much research has been done over the last four decades to understand the effect of load pulling on horse performance, but the effect on donkeys and mules has received less attention. As a consequence, we have no idea how much weight a working equid can pull. Load pulling affects a wide range of biomechanical, physiological, biochemical, and behavioral characteristics in equines, and more research is needed to advance our understanding of these factors, particularly in donkeys and mules. Quantified load pulling limits could then be used by non-governmental organizations (NGOs), policymakers, and other stakeholders working with vulnerable communities and working equids to limit excessive load pulling and improve animal welfare.

Author contributions

SB and RP were involved in the preparation of the manuscript, gave final approval of this manuscript, read, and agreed to the published version of the 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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The history and welfare of working mules in the valleys of the Toubkal massif, in the High Atlas of Morocco

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The appearance of the mule in the remote villages of the Toubkal National Park in the High Atlas Mountains of Morocco can be traced back to the early years of the twentieth century. The mule population's subsequent growth in numbers accompanied the shift away from a subsistence economy that was made possible by the appearance and growth of the mining and mountain tourism industries. This paper reviews this early history, drawing on the accounts provided by early explorers and anthropologists, before developing a mixed methods approach to evaluating the welfare of pack mules in two villages within the National Park. The first village is part of the main access route to the Toubkal, the principle summit of the National Park and therefore much visited. The second is a much more remote, under visited and less developed village. Ethnographic work, studying muleteering practice and undertaken over several years is reported here and supplemented by findings from a detailed survey of the mules of both villages. This allows the work undertaken and the lived realities of the working mule across several generations of inhabitants to be presented together with data about the primary welfare concerns identified on clinical examination. In village one, 72 mule owners and their mules were surveyed and examined. Many of these mules worked in tourism, providing a source of revenue for their families. This work was often undertaken by teenagers/young adults working their father's mules. Tethering was widely practised and evidence of tethering injuries were identified in most mules. In the more remote village, 18 owners and their equids were surveyed and examined. In this population, mules were more likely to be worked locally in agriculture, building and to collect firewood. Bitting injuries associated with the use of the traditional bit were a significant concern in both villages. The universal use of a closed shoe was also in evidence in both villages; this was associated with atrophy of the frog and hoof imbalances. The reasoning for the use of the traditional bit and closed shoe are presented and alternatives discussed.

KEYWORDS

pack mules, working equine welfare, mountain tourism, mountain development, High Atlas, One Health, wounds, bitting injuries

Introduction

The emergence of the mule's role as a beast of burden working in mountain tourism is founded on our appreciation of this species' great attributes (1, 2) as a means of transport in remote mountain environments, where road and other transport infrastructures have failed

to penetrate (3–6). These attributes prompted Alphonse Guénon (1) to propose that, if the camel is the ship of the desert, the mule is the ship of the mountains (p. 29). Today, the mountain tourism industry employs mules across the world, from the Alps to the Andes, the Himalayas to the High Atlas (5–9). Our appreciation of mules does not always extend to their care and welfare, however, for whilst we are proficient at exploiting their services, we are much less proficient at repaying them for their work in any reciprocal sense. Where lack of resources and insufficient knowledge coincides with a harsh working environment, welfare often suffers and this inattention to working animal welfare is particularly true of the mountain tourism industry in Morocco (6, 10–14), where this study is situated.

Today, in many areas of the High Atlas, mules continue to be employed to plough the fields, thresh the corn, carry fodder and, on market day, ensure the transport of his master and all goods to and from the souk (Figures 1A–D). The mule also carries building supplies (Figure 1E), gas bottles and other less traditional household items, such as beds, sofas and even fridges! During the trekking season (Figure 1F), the mule will also find employment carrying the luggage of trekkers and other visiting tourists. Mules therefore continue to play their own part in establishing themselves as essential workers and travel companions, demonstrating unrivalled work capacities and the resilience to endure great hardship. These unusual attributes have long been recognised, with Williams and Speelman (15) writing, in 1948, that:

> Those who are staunch supporters of the mule say that, in comparison with the horse he will live longer, endure more work and hardship, require less attention and feed, is less liable to digestive disorders, lameness and disease, is more easily handled in large numbers, is less irritable, and is more capable of performing work in the hands of a mediocre or poor horseman. Whether or not all these claims may be substantiated, it is a fact that the mule is well established as a work animal in those sections where climatic conditions are severe, suitable feed often lacking and horsemanship not a prevailing art (p. 2)

The remote valleys of the High Atlas of Morocco are certainly characterised by a severe climate (16), lack of grazing (17–19) and an absence of skilled horsemanship (10). The Toubkal National Park was established in 1942 under the French Protectorate (20, 21) and since then has become a very popular tourist destination (22–24), in part because of its proximity to Marrakech but also because of the appeal of climbing North Africa's highest mountain, the Djebel Toubkal. This has led to an increase in the mule population in the Mizane Valley, which leads to Imlil, principle trailhead for those wanting to climb this mountain. The Toubkal rises to a height of 4,167 m and its valleys fall steeply and are prone to flash flooding, which means that soils are virtually non-existent on the highly eroded slopes (25). Eroded sediments accumulate in the narrow valleys, aided by the creation of walled terraces to retain a depth of soil on which crops can be grown (25, 26).

The valley's human population has also grown and their economy has diversified, with fruit orchards and tourism now playing a significant role in household economics (26). The mule population are caught up in all these changes and their welfare is a product of a complex interplay of historical, socio-cultural, socio-economic, educational, geographical and other factors. This is further compounded by the challenges posed by climate change (27). Any detailed understanding of the inter-relationships between such factors and the resulting health and welfare issues seen in the local mules calls for a comprehensive and in-depth study. This paper sets out to deliver an in-depth account of some of the various threads that make up the current tapestry of life of the working mules in this part of the High Atlas.

In seeking to develop a richer sense of the complex entanglement(s) (28–31) of factors contributing to the ways health and welfare materialise for the mule, we have chosen first to present an exploration of the mule's appearance in these valleys and the working lives they have had. This is informed by insights from ethnographic work conducted within the community between 2013–2017 and an accompanying exploration of the historical ethnographic literature. The paper is thus primarily ethnographic in nature but is supplemented by findings from survey work conducted, in 2014, in two different villages (Douars). The first Douar (village) is that of Aremd, one of four villages clustered around the trailhead at Imlil, within the Mizane valley (Figure 2). The second Douar is called Tizi Oussem and is located in a neighbouring valley, that of the Azzaden. This valley is much less visited and has been less impacted by mountain tourism; this allows potential differences in husbandry, working practices, health and welfare to be explored.

This mixed methods approach allows us to consider the following research questions:

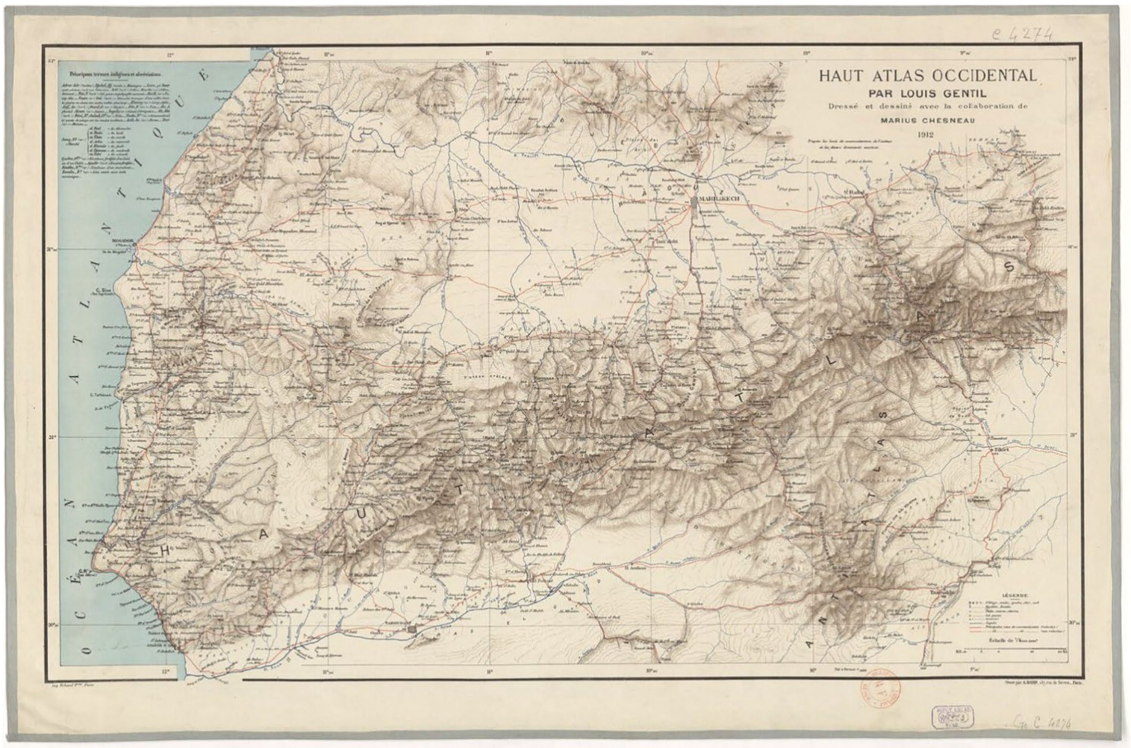
i. How has muleteering emerged and evolved in the Mizane and Azzaden valleys?
ii. What does life for a working mule in the Mizane and Azzaden valley consist of?
iii. What husbandry practices are seen in these two valleys?
iv. What health and welfare concerns are seen in these two valleys and what are the likely underlying causes?

The materials and methods that allowed these strands of data to be collected and analysed in order to explore these questions are presented next. There then follows an empirical section in which key findings are presented and discussed together, as is common practice in ethnographic work. The paper closes with an extended discussion in which we try to make sense of these elements and what they may offer in the way of insights that can help develop priorities for working equid health and welfare interventions.

Materials and methods

In this section, we provide an outline of the research approach taken in this study. This not only explicates the three data collection parts of this project and how they inform each other; it also emphasises the methodology underpinning this work. In this study the welfare of working mules is conceived ontologically as an enacted networking that arises in the moment as a result of the geo-socio-culturally situated practises of those, including the mule, who enact welfare (31). This network is a system that cannot be reduced to its constituent parts and analysed in a reductive, lytic sense for we then lose sight of the whole. It must be understood as an emergent system of relational

FIGURE 1
Traditional life in the High Atlas sees the mule working in agriculture: ploughing **(A)**, threshing **(B)**, transporting fodder **(C)** and people **(D)**. Three images are from the Aït Bouguemmez valley, the fourth **(C)** was taken above the village of Magdaz, in the Tessaout valley and illustrates the daily need for fodder to be cut and brought back to feed the animals housed within the village. **(E)** Working in construction: in the Ourika valley, motorised transport and roads are present and can be used to transport building materials. Even here the mule is still required to deliver materials directly to the building site, which may be some distance from the track. These materials may now consist of bricks and blocks, rather than the more traditional earth and stone, but the mule's versatility and utility ensure that the local populations remain as reliant as ever on this 'work horse'. **(F)** Mules in mountain tourism: the three muleteers pictured in the Tessaout valley, in 2008, have chosen to ride their loaded mules along the riverbed and have offered a ride to a tired client on the spare mule. Here spare does not mean unladen but rather unridden, for the mules are carrying all the trekking and camping equipment for a party of six trekkers, their guide and three muleteers.



Source gallica.bnf.fr / Bibliothèque nationale de France

FIGURE 2
The Western High Atlas of Morocco stretches in a curve to the south of Marrakech. The red line running north to south in the centre of the map links Marrakech to the Toubkal massif. Source: Gallica.bnf.fr (Bibliothèque Nationale de France).

elements that, together, construct the whole and are also available for deconstruction.

The three elements of fieldwork whilst presented below in succession, were in many respects concurrent as findings arising in one area prompted exploration of another area. An ethnographic retracing of a visit to an abandoned mine with a local who had worked there with his mule thus prompted this area of literature to be searched, whilst the discovery of an old account of the traverse of a remote col. prompted this journey's re-creation with a local guide and muleteer in order to experience and study the elements that such approaches make available (31). The surveying work involved daily walks up to Aremd and several day-long treks across a high col. to reach Tizi Oussem. These three approaches reflect different ways of knowing that each contribute to our understanding of the health and welfare concerns of working pack mules in the Mizane and Azzaden valleys. The constructivist methodology must be appreciated in order to see how the following methods each help us develop this richer understanding and appreciation of the contexts within which any relatively objective findings are situated.

Ethnographic walking and reading

It is widely acknowledged that any body of literature should be approached from a historical perspective when conducting a literature review (32). This does not, however, simply equate to providing a disciplined chronological account for there is a need to situate the literature within its historical contexts and to critically examine the history of the topic itself (33). Georgiou (32) proposes a series of eight questions that can help in reading into a historical body of literature (pp. 267–8) and can "facilitate a progressive appraisal of the integration of history within a literature review, one that can feed back into itself and thus allow for corrections, amplifications, new questions, and even new discoveries" (p. 267). Whilst it is emphasised that these need not be followed exactly and can be changed substantially, we have held them in mind when seeking to explore the emergence of muleteering in these valleys. In particular, we have tried not to judge previous research but rather to understand the objectives, whilst remaining sensitive to changes in nomenclature and remaining sensitive to the relations between older and more recent literature. We thus recognise that the earliest records of visits to these remote valleys arose within a disparate set of practices that predated the work of the socio-anthropologists who subsequently studied the area (34). These practices include early exploration, military expeditions, mining, and alpinism to name a few. Hassan Rachik (34) reminds us to consider the positionality, theoretical ideas and biases of the researcher and that these influence what is considered worth observing, questioning and reporting on as well as how it is reported.

For the purposes of this paper, the exploration of the literature involved not just reading into the peer reviewed literature but visiting the archives of the Club Alpin Français de Casablanca (31, p. 107) and

seeking out early accounts of expeditions[1] (in French and English) and reading them carefully in order to identify mentions (typically fleeting) of mules and other working equines and any insights they might offer on the role of mules in local communities, the care they received and their welfare. Developments that impacted on the use of mules, including mining, road building and the development of alpinism and then mountain tourism were also explored. This meant the literature review was unsystematic and exploratory, open to surprising mentions appearing in unlikely places and new insights arising as the literature was related to the ethnographic experiences on the ground. It is these we consider next:

Any ethnographic study of mule welfare in the mountain tourism industry involves journeying through time and space. Such ethnographies are, by definition, multi-sited (35), focussing both on the animal and the emergent human-animal relations and understandings that arise through and in such forms of itinerant living and their accompanying practices. These journeys unfold and are thus amenable to study. Cousquer [31, pp. 102–108] provides a detailed account of how field sites were accessed and insights gained into the history of muleteering that could then be related back to the monographs, guidebooks, articles and papers consulted. The field notes and thesis produced as a result of this have been reviewed retrospectively with a view to answering the research questions considered in this paper.

Survey

The opportunity to carry out a systematic survey of the mule population and mule owners arose when we were asked to supervise a DMV[2] research project. In developing this idea, we chose to focus on the largest of the four villages in Imlil that has been exposed to the phenomenon of the Toubkal Trail and a village in the Azzaden valley. One of the elders of the village of Aremd who has since become the President of the local muleteer association, coordinated the study ensuring all members of both villages who owned a mule participated. The work was conducted over 2 months (March and April 2014), during which time two treks over were undertaken to Tizzi Oussem for multi-day stays in the village.

The survey consisted of an interview and, in the majority of cases, a home visit and a basic clinical examination. The survey work was undertaken by a single individual who was fluent in French and Arabic and therefore able to speak to the villagers without the need of a translator. The President of the local association was available to help with checking particular terms in Berber, where such clarifications were needed. Supervision of the individual conducting the field work was provided to improve reliability but it was not possible, in the time frame, to assess inter-observer reliability. Interviews were not recorded; instead findings were recorded on paper and subsequently uploaded to a database. The interview of the owner or a member of the family sought to develop a picture of the mule's life including:

i. When the mule was purchased/length of ownership?
ii. Where the mule was purchased and at what age?
iii. Who worked the mule?
iv. What work was undertaken and in what proportions?
v. Foot care including date of last shoeing, frequency of shoeing, and foot care.
vi. Dietary information – food fed and amount fed.
vii. Watering practices.
viii. Where stabled, size of stable and stable management?
ix. Rest periods and what this consisted of including whether allowed free time?
x. Tethering practices.
xi. Contact with other mules and livestock.

The clinical examination sought to establish/confirm:

i. The mule's age (<5 years/5–15 years/>15 years).
ii. Sex (male or female).
iii. Coat colour (bay, grey, or chestnut).
iv. Weight, following the protocol developed by Kay et al. (36).[3]
v. Attitude when approached, a hand placed under chin and walked.
vi. Body condition score (0–5) (37).
vii. Conformation of the midline (back and withers).
viii. Presence of wounds.
ix. Presence of dental hooks.
x. Foot examination.
xi. Lameness.

Answers were captured in a notebook and subsequently uploaded into an Access database. Data was cleaned, categorised and analysed quantitatively in Python 3.7. Plots were produced using the seaborn package (38) and Chi square tests of association between variables carried out using the scipy package (39) *chi2_contingency* command.

Ethics statement

The survey part of this study was planned and undertaken at a time when the IAV had yet to establish their *Comité Ethique* and so the study could not be submitted for ethical review. The analysis of the data collected that we have undertaken for this paper is, however, a prospective study using an existing data set. This analysis was therefore submitted to the University of Edinburgh's Veterinary Ethical Review Committee (VERC), within the Royal (Dick) School of Veterinary Science, for approval in 2023 (Reference: 107.23). The ethnographic part of this study is reported retrospectively here. The original study was approved by the Ethical Committee of the University of Edinburgh's School of Geosciences.

Results

Relevant elements of the ethnographic study are presented first, followed by key results from the survey work. The former will, by its

1 From references in secondary sources, Google Scholar and online archives of turn of the Century texts.

2 Docteur de Médecine Vétérinaire.

3 Calculated from the girth and distance between the elbow and tail base.

very nature be discursive and will help to situate and contextualise the survey results. These too are discussed as this is consistent with the narrative sense-making developed here.

Retracing the early history of muleteering on the Toubkal

The emergence and evolution of muleteering in the High Atlas of Morocco can be discerned in the writings of early explorers. In Morocco, the mountainous interior fascinated many such explorers, including Buffa (40), Cuninghame-Graham (41), Harris (42), Rohlfs (43), and de Foucauld (44).[4] Travel and tourism are, however, dependent on safety, with early travellers being provided with armed escorts or guides to ensure safe passage (41, 45, 46). According to Boujrouf et al. (47, p. 69), tourists only arrived in the massif after its pacification. The High Atlas has long been a mountain fortress, serving as sanctuary and refuge to those who lived there (48, p. 141) and resisting the incursions of both alpinists and occupying forces. Unsurprisingly, it was one of the last parts of Morocco to be mapped, with some areas remaining blank until the 1930s (49, 50). As recently as 1917, 5 years after the creation of the French protectorate, only those areas in which topographers could venture as part of a military column had been surveyed, leading the topographer Théophylle-Jean Delaye to describe these as completely unknown and closed to Europeans (49, pp. 3–4).[5]

According to Boujrouf et al. (47, p. 73), the Atlas was, during the 1920–30s appropriated by alpinists-*cum*-scientists. Their various efforts paved the way to the creation, in 1942, of Morocco's first National Park, the Toubkal National Park (24, 54) and the emergence of mountain tourism from the 1920s onwards (55, pp. 224–225). The history of mountaineering and subsequently mountain tourism in the High Atlas can be traced back to the pioneering activities of a small group of alpinists and the founding, in 1922, of the Moroccan High Atlas section of the French Alpine Club (CAF). This led, the following year, to the first ascent of the Djebel Toubkal (55). Somewhere in all these explorations mules started to make an appearance.

An 1892 print, by Caton Woodville, entitled "The Mountain Path" is one of Woodville's scenes without occidental tourists from his time in Morocco (56, p.93). It depicts the narrow mule paths that mules and their riders are likely to have had to contend with (Figure 3). One of the earliest accounts of travel into the challenging terrain that lies above Aremd was written in 1919 by Paul Penet (45) and tells of his traverse of the Tizi n Tarharat (3,460 m) in 1917.[6] This is one of a number of high cols in the Toubkal area and is shown as the main route from Marrakech south across the High Atlas, on the 1932 map by Louis Gentil (Figures 2, 4), passing through Asni and Aremd before crossing over the Tarharat to reach the remote village of Tissaldi in the Tifnout valley. Penet's account of his group's voyage is insightful for it tells us much about the terrain, how local villagers travelled across it and what was transported. He writes (45, p. 8):[7]

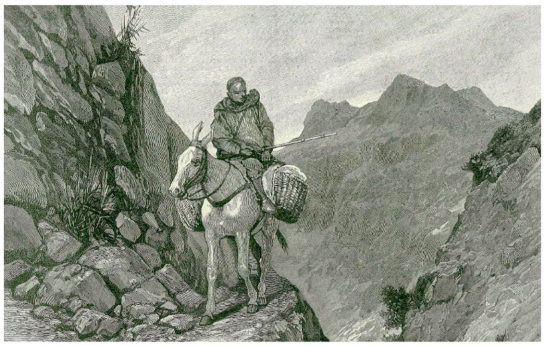


FIGURE 3
The mountain path by Caton Woodville (56) illustrates the narrowness of mule paths and the steep and exposed terrain mules work on.

> We climbed little by little; everyone dismounted except Si Abd en Nebi, the envoy of Si el Madani, who remained on his horse. He was all pale, altitude sickness and palpitations made walking impossible for him.
>
> At 3,100 m we stopped, man and beast needing a rest. It was cold. For the first time our lungs drew in the delicious cold air that fell towards us from the ridges. A spring offered us ice-cold water (6°). At the edge of the path we saw several dry stone shelters where travellers would seek shelter from snow storms or for the night. They also serve to shelter livestock as the people of the Tifnit do not hesitate to drive their cattle across the Tizi – Tar'ret to sell them in Marrakech.
>
> Two men on foot joined our group: bare headed, in rags, one with goat hair slippers on his feet, the other sandals made from walnut wood. They were returning to the upper TIfnout after 6 days away. They had been to Moulay–Brahim (a 3 days walk) to buy two measures of maize that they were carrying on their backs. These people are incredible.

His account of this journey emphasises how unsuitable this route (and the terrain more generally) is for horses, that the local villagers travel everywhere on foot and that mules and horses are a luxury (45, p. 13):

> The horse of Si Abd en Nebi lay in a neighbouring encolosure. Laminitic and colicing, he was in a bad way. The owner appeared disinterested and we found ourselves insisting the horse be rubbed, covered and treated.

4 See Rachik (2012) (34) for an exquisite review of the different ways in which travellers have come to know Morocco.

5 Delaye was put in charge of the aerial photography section of the '*Service géographique du Maroc*' in 1926. His pioneering work, first in the Rif (1925–26) and subsequently in the regions of Ouarzazate and the Drâa valley (1932) and then the Sagho (1935), allowed the occupying forces to pacify the mountain tribes [46;47]. In 1937, he produced a map of the Toubkal massif at a scale of 1:20,000 (51–53).

6 The Tizi Tar'ret. as he spells it had according to Penet (45, p.10) already been crossed first by Von Fritsch, then Gentil but had seen Brives renounce his attempt due to the poor reception ("mauvaise disposition") of the people of Aremd.

7 Translated from French by the author (GC).

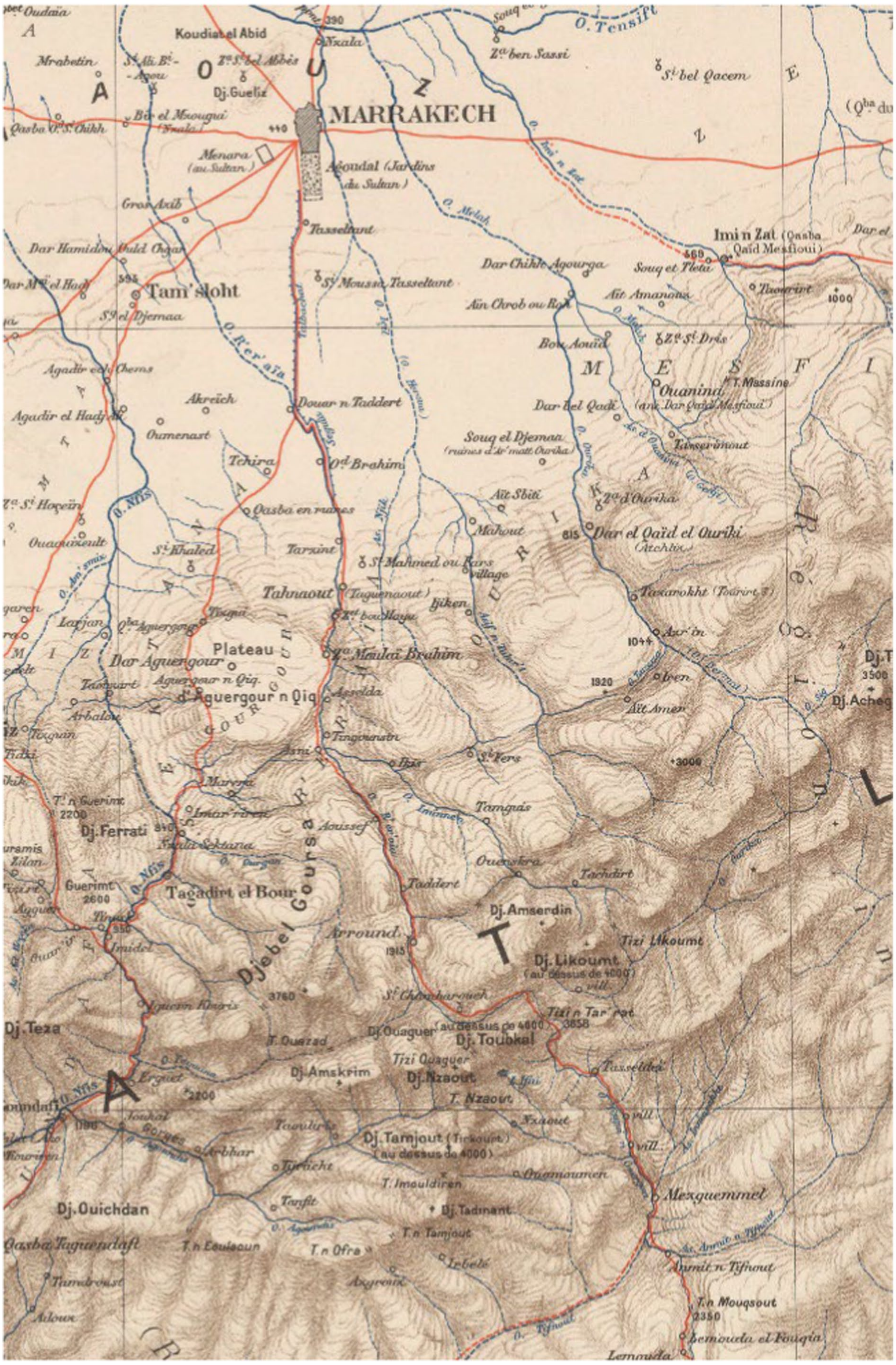


FIGURE 4
A close up of Louis Gentil's (45) map of the western High Atlas shows a red line descending from Marrakech, passing though Asni and Aremd (spelled here Arround) before turning east at Sidi Chamharouch to cross the Tizi n Tarharat (spelled here Tizi n Tar'ret). The village of Tizi Oussem and the valley of the Azzaden are not indicated on this map. The Source: Gallica.bnf.fr (Bibliothèque Nationale de France).

… After a day as hard as yesterday's, our animals had earnt a rest. Our schedule fortunately meant that today's stage was very short.

Aguezrane where we were due to camp that night was no more than 8 km as the crow flies. But the path we took was so abominable, hugging at times the granite mountainside then snaking in the bed of the torrent when it was not in the torrent itself, that our journey took three hours. To say that this path serves the villages that are strung the length of this valley is an exaggerated euphemism; and it is precisely around the villages that the paths are most difficult. We realised that the habit of these mountain people is to travel on foot. Owning a mule, let alone a horse is an insolent luxury that only the chiefs can afford. Our caravan of nine equines, ten with that of the chief who came out to meet us, is so unusual that the terraces were thronged with curious faces as we passed.

The Tarharat is one of a number of cols close to or above 3,500 m surrounding the Toubkal, together with the Likemt (3,555 m), the Ouanoums (3,664 m), and the Ouagane (3,750 m). As the lowest, it was the route of choice for villagers from the Tifnout heading to the souks. Penet (45, p. 21) emphasises that the difficulty of the paths is such that the principle exports from these valleys are walnuts and livestock and these are sold in Marrakech and Taroudant. Wool is not exported for it is consumed locally. Over the next few decades, these isolated villages were increasingly influenced by the outside world, prompting Dresch and Lepiney (55, p. 38) to write that:

> The villages lost in the depths of their valleys no longer live in isolation, in a self-sufficient subsistence economy. The markets and the distant big city are a permanent temptation. And prices change. Why? The mountain inhabitant knows little of the national or international economies. The reality is that he has to spend more, no matter how much he argues over each and every penny; more sometimes than his meagre budget allows for. And so he must sell more and more.

The common thread in these different historical strands is that they are all related to a fundamental imperative – that of selling to raise money… But what can these mountain communities sell? Historically, they have sold walnuts, almonds, livestock, walnut roots and a range of other saleable commodities. Iris bulbs became valuable once they became sought after by the perfume industry. And those that had nothing to sell would try to sell their knowledge or their labour or, if they were lucky enough to have a mule, that of their mule. Fundamentally, these communities have to find things to sell. And then they have to find buyers…

During my field work, I crisscrossed these high cols, paying close attention to the terrain, to the challenges it posed, to how local muleteers worked their mules over this ground and to what was being transported and why (Figures 5A–C).

The nature of the saleable goods carried has extended from walnuts to include almonds, iris bulbs, walnut tree roots, and fruit including apples and cherries and other produce grown in these high valleys. Fodder and firewood can be carried by mules but it is often the womenfolk who will undertake this work. The main reason for mules to appear in these high valleys can be traced, therefore, to the appearance of first mining and then mountain tourism. Penet (45, p. 15) tells of a local Jew who shows them samples from a secret local mine that he is willing to sell to them. According to Moret (57, p. 262) Morocco had a reputation for being a fabulous mining Eldorado. This was largely based on the simple testimony of indigenous people who were thus transmitting the memories of the Roman and Portuguese miners, of old.

The development of mining operations in and around the Toubkal massif was more than just folkloric. There was living memory and experience of it on the ground. Mohamed was born in 1950 in Imlil, the village just below that of Aremd. He told of how he went to work in the mines at the age of fifteen because his father was unable to work. He did not want to work in the mine at Tadart, situated high up above the Neltner refuge (3,207 m) on the flanks of Aguelzim (3,680 m). This mine was reputed to be very tough as there was no mule path up to the mine. Consequently, the miners would carry the 50 kg sacks of mineral down to the Neltner refuge where they were loaded onto mules. He said that the miners might earn seven dirhams a day (approximately £0.53).[8] The mules would have had to carry their load down to Imlil, which only became accessible by piste when this was completed in 1956 (58, p. 24).[9]

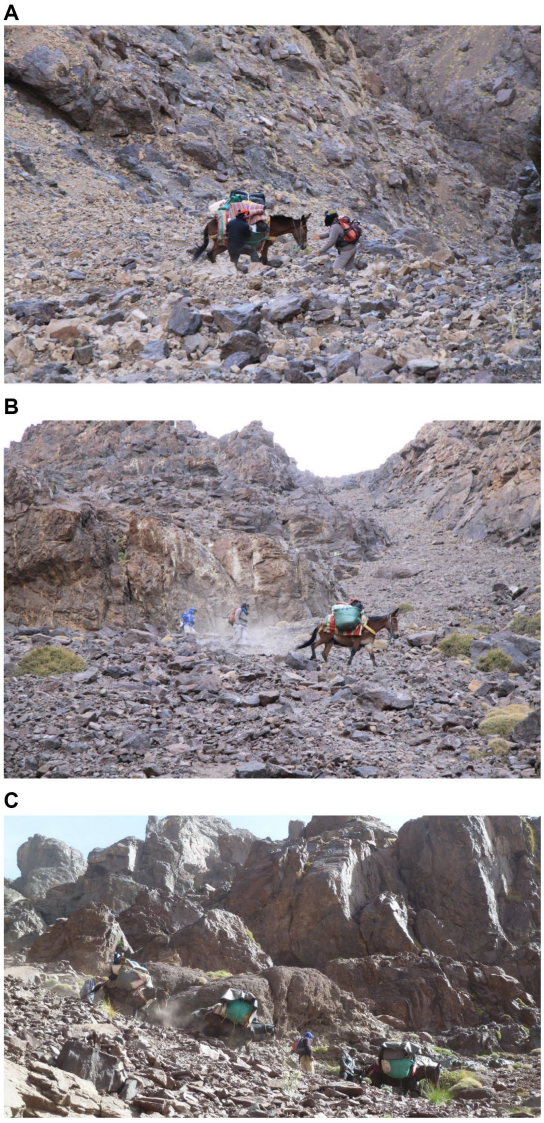


FIGURE 5
(A) The guide and muleteer intervene to rebalance the mule's load as she descends the upper section of the scree at the top of the Tizi n Tarharat. **(B)** Looking back up the Tizi n Tarharat: the nature of this steep ground and rocky scree can have a heavy impact on the mule – giving rise to pack saddle sores, tendon strains, wounds and foot injuries. **(C)** A train of mules and muleteers working on a trek descend towards the Lac d'Ifni in the Tifnout valley, having crossed the Tizi Ouanoums. This ground is steep and unforgiving and the loads are heavy. All these mules are worked in traditional bits. Note how the mule handler in the centre of the image has created a noose around the neck of his mule to pull her forward, whereas other handlers are working their mules from behind.

8 At an exchange rate of 13 dirhams to £1.

9 The completion of the piste is reported in the news section of Montagnes Marocaines, the journal of the Casablanca section of the Club Alpin Français.

Another local informant who had worked in the copper mines above Aremd took me to see where he worked and explain what was involved. Omar's story is typical of someone who grew up in the valley. As we walked the old and vanishing mule paths together, he told me how he had spent much of his early working life on the steep and unforgiving mountainside above the village. We were heading up to visit the abandoned copper mines above Tidli.[10] He had worked there as a muleteer for 3 years in succession (1991–1993), leaving at 5 am to arrive at the mine for 7 am. He would often give one of the five miners that accompanied him a ride on his mule. He would then load two sacks of mineral ore, each weighing between 75 and 100 kg, onto his mule's back and head back to Imlil, where he would arrive at about 11 am. There he would unload and receive a chit for his work. At the end of the month, when the lorry arrived from Marrakech to pick up the mineral, he would be paid, receiving a meagre 17 dirhams per day.[11]

> I asked him about the load on the mule and whether she suffered any injuries. He was of the opinion that, in those days, the mules were much stronger – partly because they were better fed. There was much more barley being produced in the valley in those days and this suffered with the switch to fruit trees.[12]

The arrival of apple and cherry trees and the high cost of buying in barley has, he explained, meant that "some owners underfeed their mules and some are even reduced to giving them spaghetti!"[13] [14]

The paths we explored together had all but disappeared, reclaimed by the hillside as one rock slide after another had conspired with broom bushes to render the evidence of the mules' passage invisible. Omar, however, could retrace the path for it was etched in his memory, as it had been in his mule's.[15] Thus, as we followed what was once his daily commute, a picture emerged of the precarious existence these men had carved out for themselves.

> I learnt that Omar's father had worked a lot in this mine and that they had worked together. This was at the end of the mine's working life. The miners had used explosives and drills to work the mine and, impressively, there had been no accidents. The only accident occurred after the mine had closed when a local guide fell into one of the shafts and suffered injuries from which he was to die.[16]

I asked Omar why they chose this work and heard him say

> … in those days there was no choice. There were very few fruit trees and little or no work in tourism. When the mine closed in 1994, … he went back to shepherding for a few years before … finding work in tourism as a muleteer and then, eventually, as a cook.[17]

At the mines, Omar explained to me that the mules

> … once loaded, would not come back along the path we had just tried to follow. This, he said, was too steep. Instead they climbed up to the col above us (the Tizi Oufzdad) and from there dropped down to a much better path that contoured round the mountain.[18]

We followed this route, climbing over the col. before dropping to the *azibs* (shepherd huts and sheep folds):

> We descended to the azib – a veritable scree run over difficult ground, all cut up by water channels. He explained to me that he had spent years of his life tending sheep and goats up here and that the azib belonged to his family.
>
> Today, nobody in Achayn wants to shepherd. He said it wasn't clean ('propre') and the life was too hard. They now employ a shepherd from Tizi Oussem to come over and look after the sheep; his family now only keeps one sheep at home.

Thus, as one source of income closes, another opens. Today, Omar has managed to secure safe, regular employment as a chef, at a local hotel. This means he has a guaranteed income and is in receipt of social security.[19] He is also able to supplement this income by renting out his two spare rooms to visiting Moroccan tourists, earning him 150 MAD per room, per night. Life is thus considerably better than it once was and he will be able to send his children to school.

Omar's story is echoed by others of his generation. In Aremd, there are only a few old shepherds left, no one is continuing the tradition; the shepherds stepping into their shoes come from neighbouring valleys – and in this case, specifically the second village in this study, Tizi Oussem. As Fabrice Cuzin explains when talking about generational change in the villages: "The inheritance of a way of life is broken; … the old no longer serve as a reference for the next generation. There are no apprenticeships".[20] Michèle Salmona (59, pp. 93–97) emphasises the importance to animal welfare of life-long apprenticeships in peasant societies: Whether speaking of the Peul or the French, the transmission of animal knowledge from one generation to the next necessitates the selection of those with an aptitude for this work and the development of a slow, gentle, patient

Until that point, the amine who was contacted to organise mules for a trek in the valley was located in Asni.

10 Tidli is the name given to the area above Aremd. According to Hajj Morris (personal communication 31/08/14), the name of the mine above Aremd was 'Couchar', whilst the bigger one above Tamatert was called 'Talat n Taghsen'.

11 Equivalent to £1.30 (at an exchange rate of 13 dirhams to £1).

12 Field Notes 18, page 8 (30th August, 2014).

13 Field Notes 19, page 19 (2nd September, 2014).

14 For further information on the introduction of fruit trees to the valley see Funnell and Parish (19).

15 Mules can often follow familiar paths in the dark of night, so well do they attend to their passage. The history of mining is also etched into the memories of the old men of the valley, many of whom were reduced to seeking work there when they were not needed in the fields or to tend their livestock.

16 Field Notes 19, page 24 (2nd September, 2014).

17 Field Notes 18, page 8 (30th August, 2014) and Field Notes 19, page 30 (2nd September, 2014).

18 Field Notes 19, page 24 (2nd September, 2014).

19 Recent changes to Moroccan legislation mean that this covers not just his wife and children but also his parents.

20 Field Notes 18, page 4 (29th August, 2014). Interview with Fabrice Cuzin, consultant biologist, ex-manager of the CAF refuges and instructor at the CFAMM. Translation: «La transmission est. cassée … les anciens ne représentent plus de référence pour les jeunes! … Il n'y a pas d'apprentissage!»

disposition and fortitude in the face of hardships and isolation. This transmission and, in particular, the affective component, is lost when children go away to school or attend college later in life to learn about the technical (*I-It*) aspects of animal production (59, p. 97). The young of the valley are all striving to establish new lives and forge new paths for themselves and their families. But how does one escape from poverty? What mules and mule welfares does this absencing enact?

Where historically artisanal mining had offered opportunities to earn hard currency (57, 60, 61), today other opportunities have emerged. Those who complete their baccalaureate can go on to university and can apply to the guide school (57) with a view to training and qualifying as a mountain guide. The ambitious aspire to set up agencies and partner with a foreign agency who will send them clients. Many set up gîtes, adding to the supply of accommodation that has seen Imlil offer nearly as many B&B bed nights (47) as Casablanca (57) on TripAdvisor![21] This uncontrolled construction programme (62) has, arguably, blighted the peaceful character of the valley in what Goodwin (63, pp. 18–22) has described as a "tragedy of the commons." Those with language skills but no qualifications can find work as receptionists, waiters, drivers and faux guides. Options are limited, however, for the young, unmarried men, who have not completed their schooling. During harvest time, employment can be found bringing in the walnuts, cherries and apples.[22] This, however, does not provide a regular income stream. The lucky ones find work locally in shops, hotels, restaurants and cafés; many though find themselves leaving to find work in the cities. Some travel down to Dakhla to find work as paid agricultural labourers, others seek work in Casablanca and other cities or further afield.

De Sinety captures the paradox of the rural exodus that sees young Berbers of the Atlas descending to the cities where they find themselves shipwrecked in an alien world where capitalism and individualism hold sway, a world that the visiting tourist is anxious to flee:

> They had to leave their valleys in the High and Anti Atlas that were no longer able to feed them. Washed up in noisy, aggressive and polluted cities, far from the solidarity of their village communities, they are forced to confront the apprenticeship of an urban capitalist society with its emphasis on individualism.
>
> A minimum material need for subsistence leads these young Chleuhs to the city, whilst a spiritual quest leads privileged westerners to leave them. Our paths cross, our motivations are opposed, but we can still meet. (64, pp. 7–8)

Spaak (65, p. 228) similarly comments on the socio-cultural insularism that renders it difficult for the people of the High Atlas to leave their villages behind and venture down onto the foreign, anonymous plains where life is easy and a man's bond no longer has the same value. Necessity; however; forces them to leave to find work (66). The exodus of over 50% of menfolk from these communities is; according to Spaak (65, p. 230), as grave a concern as desertification. He highlights the developments in agriculture that could help stem this "haemorrhaging," whilst also referring to the promise offered by exploiting other resources including minerals, walnut wood and tourism. Since then, the High Atlas has been transformed by the development of opportunities to earn hard currency from fruit farming and mountain tourism[23] (26, 61) and from the attempts to turn local resources into heritage objects (68, 69).

This has not, however, been without its problems for the environment (24, 61) and for communities, which once cohesive and well-ordered are increasingly divided by competition (68, 69). Tourism has thus allowed those who became guides and gîte owners to make their fortune. But what of those left behind? The unlucky ones – those from poor families, with little or no education – are typically reduced to working as muleteers. Those who have escaped this fate, recognise how lucky they are. A faux guide and driver whose brother still works as a muleteer acknowledges the extent to which muleteers are exploited by the industry:

> The muleteers do a lot … I don't mind if they only pay us 200 dirhams and the other 50 goes back to the muleteer … because they do most of the things … what am I doing? It's nothing I take my bag and go walking, I have nuts, water, oranges, I can stop wherever I want and chat with the guests. But the muleteers have to charge the mules … walk so quick to pass you and you have to find them already making your lunch and then they have to charge the mules and keep going again to reach the final destination. Once he gets there he has to feed his mule, wash, make the dinner. They are doing lots of things for nothing. The payment for the muleteer is nothing at all. Those people deserve more than that. They deserve double price; they deserve even 180 or 200 dirhams.[24]

The muleteer's unenviable status places them just above shepherds: at the bottom of the pile. Some spend hours waiting for clients, earning as little as 10 MAD to carry suitcases from the village up to one of the hotels or gîtes. The mules wait too; standing tethered, with their pack saddles on, denied food and water, unable to escape the heat and the tormenting flies.

During the summer, the influx of Moroccan tourists seeking relief from the heat of Marrakech (71, p. 6) provide a stream of potential clients and opportunities to earn 100 MAD for doing the trip to Sidi Chamharouch, potentially 200 MAD if two trips can be squeezed into a day.[25] These 'touristes internes' often negotiate hard and show little concern for mule welfare. On a trip, up to Sidi Chamharouch, Hamid, a muleteer from Aremd, said:

21 Details for B&Bs and Inns checked on the 6th September, 2017. This excludes hotels.

22 In the case of walnut harvesters, this is recognised as dangerous work, with workers having to scale the trees and move through the canopy to knock walnuts to the ground. As a result, it attracts a daily rate of 300 Mad per day with the walnuts selling for 200–50 MAD per thousand. By comparison, a daily rate for apple harvesters is 100 MAD.

23 Within this, it is important to distinguish between three forms of tourism: that involving foreign trekkers, that involving locals escaping the city and that involving locals undertaking pilgrimages (67, p. 6).

24 Interview with Lahcen, April, 2015.

25 These rates are from 2014–2017. With the creation of a new muleteer association for the valley, covering the villages of Aremd, Mzik, Aït Souka and Imlil itself, a revised fixed set of rates have been introduced.

> … foreigners were much readier to think about the mule (they liked animals more). He explained this by saying that they would save their scraps for the mule. By contrast, Moroccan tourists would often say that his mule was no good and ask him to hit it in order to make it go faster … He refused to do so and said: "Je ne l'ai pas trouvé dans une boite de Vache Qui Rit".[26]

Muleteers seeking such work also must contend with the influx of men and boys from other valleys, desperate to secure work and willing to undercut the typical daily rate.

> The muleteers has [sic] to be all together. If they are not together they are not making any deal. … If Imlil decide to go for 150 dirhams, but Tachedirt, Oukaïmeden, Ourika they can still come to work for the same price. They have to send a letter to each village in the Atlas who has muleteers who work with tourists … if anyone move without this price … then from there, there can be …. It will be difficult but if they work with each other and behave each other they can get to that point.[27]

During the summer months, many muleteers purchase an old worn out mule and flog her hard through the summer to scrape a living. This can mean denying her rest during that time, then selling her on to avoid paying to feed her through the winter.

> On the track to Aït Aïssa, and again on the climb up to the Tizi n'Oudite, Hamed told me about the challenges of finding work in Imlil. According to him, in his village of Aran, there are only two people who have proper jobs … They are employees … they have a certain income. The other men of the village have to find work where they can. For him, this means working hard with a mule through the summer and putting some money by to get him through the winter. He has worked in the cities (both Marrakech and Casablanca) – in pizza restaurants. In Imlil, the men have little to do, however, other than the seasonal work that agriculture and tourism throws their way.[28]

Hajj, a village elder, explains the problems this can give rise to

> Tu connais la région, tu connais les gens. Tu vas trouver il y a quelqu'un qui est pauvre … acheter un mulet pour 3000 dirhams. … Un mauvais mulet … Mais qu'est-ce qu'il fait parce qu'il a besoin pour le travail … pour gagner pour la famille. Lui, il pense seulement gagner un peu d'argent. Il part sans penser pour la mule. Tu connais les mules qui sont mort dans la montagne c'est les mules pour les pauvres 'man' qui a acheté la mule pour 3,000 dirhams, 4,000 dirhams.[29]

This is life on the margins. The precarious existence led by those struggling to eke out a living is, largely invisible to the visitors and agencies; the consequences on the mule are therefore viewed not through the lens of phoric hardship described by Salmona (59) but through that of the disapproving tourist and employer. Such has been the challenge of understanding the welfare of equines in the Arab world, since Daumas (72), in the mid-1800s, cautioned against judging too quickly:

> … beaucoup des personnes ont conclu que ce peuple n'avait aucune connaissance des vrais principes hippiques; elles lui ont même refusé tout amour du cheval. C'est qu'elles n'ont point voulu réfléchir que, tantôt pour sauver leurs familles, tantôt pour conserver leurs biens, et souvent pour obéir aux lois de la guerre sainte (djéhad), ces mêmes Arabes … étaient forcés de se servir de leurs chevaux en raison des besoins qu'ils éprouvaient, des circonstances qui les dominaient; mais ils savaient parfaitement qu'il eût été préférable de ne point agir ainsi.[30] (72, pp. 151–152)

These then are the geo-historical, ecological, socio-economic, and cultural contexts within which any exploration and evaluation of working mule welfare in the valleys around the Toubkal are situated. We now turn to and consider a selection of findings from the villages of Aremd and Tizi Oussem to further deepen our understanding or working equine welfare.

The working life of mules and muleteers in Aremd and Tizi Oussem

The human population in Aremd (AR) is approximately 1,900–2,000 people, whilst in Tizi Oussem (TO) it is estimated to be 600–670.[31] The survey documented at least 72 mules living in Aremd. All mules were female, except for one gelding. By contrast, in Tizi Oussem (TO), a much smaller village, sixteen mules were documented, all female; as well as two male donkeys (Table 1). Owners attributed this choice to the fact female mules are calmer, more patient and easier to work with. Mules in both villages were predominantly aged 5–15 years with only five and three mules over the age of fifteen reported in each village. In Aremd, seventeen owners had owned their mule more than 10 years and three for close to 20 years (Figure 6). Mean length of ownership was 5.5 years in AR and 3.9 years in TO. This reflects the typical working age of mules working intensively on this terrain – historically in the mining industry and today in the mountain tourism industry. Mules who

26 Field Notes 4, page 5 (25th March, 2014). Translation: "I did not find her in a box of Laughing Cow cheese."

27 Interview with Lahcen, April, 2015.

28 Field Notes 43, page 23 (21st October, 2015).

29 Field Notes 35, page 11 (3rd June, 2015). Translation: "You know the area, you know the people. You will find someone who is poor … who buys a mule for 3,000 dirhams … a bad mule … But what does he do? He needs a mule to find work … to keep his family. He is only thinking of earning a little money and he goes without thinking about the mule. You know the mules who have died in the mountains. These are the mules of poor men, the men who have bought their mules for 3,000 dirhams, 4,000 dirhams." A good mule can, by comparison, be expected to cost in excess of 15,000 dirhams.

30 Translation: "Many have concluded that this people had no knowledge of equestrian principles, refusing them even all love of the horse. This results from a failure to recognise that to save their families, to preserve their property and often in obedience to the laws of Jihad, these same Arabs … were forced to use their horses according to the needs they were subject to and prevailing circumstances but they knew perfectly well that it was preferable not to act thus."

31 According to the village associations.

10.3389/fvets.2023.1256501

TABLE 1 Differing demographic data of mules between mules in Aremd and Tizi Oussem.

		AR	TO
Sex	Female	71	16
	Male	1	2 (donkeys)
Age	<5 years	9	2
	5–15 years	56	13
	>15 years	5	3
Bought out with the Douar	Yes	6	8
	No	66	10
Mule handler	Owner	57	11
	Owner and family	2	5
	Owner's family	10	2
	Owner and others	2	0
Mules working in construction	Yes	3	3
	No	28	0
Mules engaged in tourism as their only work	Yes	12	0
	No	18	3

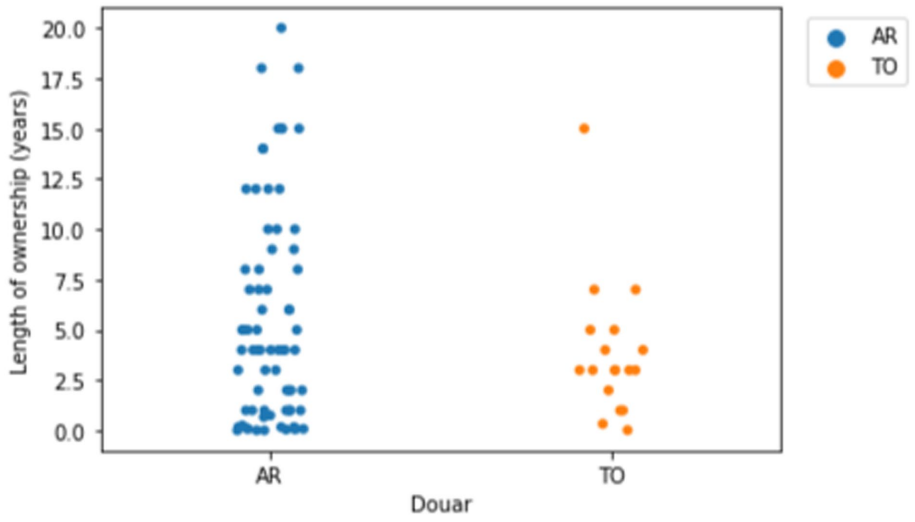


FIGURE 6
Strip plot of length of ownership in years by Douar (village). Mean length of ownership in AR was 5.53 years ($n = 72$) and in TO was 3.85 years ($n = 18$).

are no longer able to earn their keep are sold to work in the cities. A dignified retirement is not an option and owners recoup the monetary value of a mule with little thought as to how their mule might end her days working in the cities. This is a serious welfare concern for these aged mules move from carrying loads to pulling carts on busy city streets.

Mules in TO were significantly more likely ($\chi^2 = 14.30$, $p = 0.0064$) to be bought from within the village than mules in AR, where 91.7% of mules were bought from outwith the Douar (Figure 7A), with the market in Asni the most common purchase place (38 mules). Mule owners had also travelled to more distant souks, including those of Marrakech (3), Amssouzart (4), Setti Fatma (3), Agoundiss (3), Imintanout (3), and Tifnout (5) to purchase their mules. In TO, 55.6% of mules were bought from outwith the Douar, the most common purchase place was TO itself (8 mules), however. This reflects the fact that there are no horses in either valley and no mule breeding therefore occurs in the area.

Bay mules appeared to be more common or popular than grey mules in Aremd, where a single chestnut mule was identified. The weight of mules ranged between a minimum of 192 and a maximum of 397 kg (mean in AR was 259.8 kg s.d. 41.4 kg, in TO was 211.1 kg s.d. 44.4 kg). The significance of the low weight and size of the mules may reflect a preference locally for smaller mules when working in steep terrain. This data is also helpful as a guide for establishing weight limits where responsible tour operators develop mule welfare charters (13). Body condition score was generally low in both villages

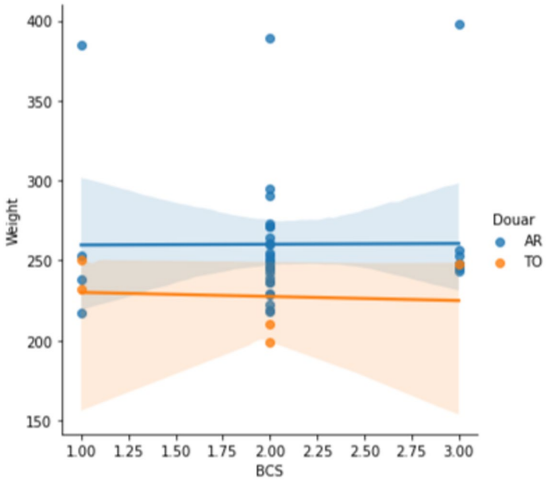


FIGURE 7
Regression plot illustrating relationship between BCS and weight of mules with a linear regression line per Douar (village). Mean BCS (on a 0 to 5 scale) in AR was 2.1 ($n = 72$) and TO was 1.8 ($n = 18$). The regression lines showed no close relationship between BCS and weight.

(Figure 7) which may reflect the lack of grazing, access to food and workload as well as other causal factors including poor dentition. Poor dentition, including the appearance of dental hooks, is likely to arise from the lack of grazing opportunities and the emphasis placed on barley in the diet.

Generally, the owner or one of his sons worked the mule (Table 1); it was rare for someone outside the family to work the mule but this did occur where the mule was owned by a village elder, willing to lend out his own mule. The relationship the handler has with the mule they work is also affected by other factors, including the fact that adolescent boys today are away from home at school and this reduces the time spent developing a relationship with their family's mule. They may then find themselves working the mule in school holidays and on weekends or when they have finished their schooling without having established a connection. Poor relations can be further aggravated where muleteering is not their preferred line of work and resentment is taken out on the mule (31). This is part of the break in inherited relational practice referred to earlier. The relationship that can arise when an owner owns, values, knows and appreciates his mule is not the same as that arising when a youth who has not invested his own funds and self in the mule finds himself having to work the mule.

Homecare was typically provided by the women of the family[32] when feeding the livestock (cows and sheep) living with the mule. No mules in either village had free access to water at home. Water was either made available when the mule went out to work or brought to them by bucket. Grain is typically fed twice daily in a nose bag made from plastic flour bags and is never soaked. In Aremd, some grazing is available above the village and mules are taken up there in groups during the winter months when work in tourism ceases. Many mules are, however, fed at home with womenfolk heading out every morning to scythe, collect and carry back fodder for all their livestock. This task is repeated again in the evening. They do so, bent double under the load for they do not work with the mules. These cultural norms are reflected in the title of a recent book on hidden injustices in Morocco: "Dos de femme, dos de mulet" (73). Montanari in her work on the future of agriculture in the High Atlas highlights the extent to which women have to work long hours collecting fodder for their animals (17, pp., 53, 54, and, 57, 58) and the challenges of sourcing fodder during the winter months. These practices and challenges were common to both villages in this study and can lead to mules being sold before the winter months to avoid the cost associated with feeding them when they are not working. Alfalfa is often fed to milk-producing cattle but its production locally is very limited and it is not offered to mules, who are primarily offered chopped straw as fodder. The use of straw and, more generally, cereal crop by-products as the sole feed source for draught animals is a common practice in many rural communities in the Mediterranean and Balkan regions. These feeds are characterised by poor nutritional value, due to the high content of indigestible fibre and this can compromise work efficiency. In the long term, the feeding of imbalanced, cereal straw-based diets can lead to poor health and metabolic disorders, such as increased calcium removal from the bones by oxalic acid, which is particularly abundant in cereal by-products (74). In Aremd, straw is largely brought in by lorry and this is coordinated by the local muleteer association. This practice has arisen because of the large number of mules and because the terraces that would previously have been used to grow wheat and barley are now largely given over to apple and cherry production (19).

Work undertaken by mules in both villages consisted of transportation of building materials, firewood, manure and agricultural produce, other household items as well as work in tourism. Mules in TO were significantly more likely to be used for construction work than mules in AR ($\chi^2 = 15.35$, $p = 0.0040$). All animals in both villages were used for tourism purposes, but none of the mules in TO were used exclusively for tourism purposes whilst work in tourism was the most important form of work in AR, with some mules only used in this sector (Table 1). Two forms of tourism work are recognised. The first involves local work and caters for visitors wanting to undertake local excursions. The local muleteer association operate a system whereby each of the four villages in Imlil are rotated for work; this means mules can expect to work 1 day in four. The second involves multi-day trips into the high mountains. This work is coordinated by the amine for each of the four villages.

Wounds and other injuries

Wounds were identified as a significant welfare concern in both villages (Table 2) and were, in all cases, associated with part of the tack – either the pack saddle, the tethering system or the bit and bridle. In Aremd, 74.4% of mules had evidence of fresh or old wounds; by contrast, 55.6% of mules in TO had fresh or old wounds.

Wounds relating to the pack saddle were identified at the level of the withers, tuber ilium, elbow, back of the thigh, dorsal midline,

32 Who often do not pursue their studies after their junior school years because going to the college requires them to travel to Asni, the nearest town.

TABLE 2 Tack practices and wounds in mules between Aremd and Tizi Oussem.

		AR	TO
Wounds present	Yes	29	2
	No	11	4
Bit type used	Traditional	19	15
	Modern	11	1
	Both	2	0
Pack fit	Tight	20	2
	Not tight	12	2

FIGURE 8
Mule tethered in Aremd in outdoor stabling area. Note the use of a single thickness of nylon rope and the loop of very thin nylon string on the ground that has also been used as a tether. Note also the extensive depigmentation of the hair over the pastern and cannon bone reflecting deep trauma to the dermis.

under the tail and the girth band. Potential factors that might predispose a mule to packing injuries include poor body condition, poor conformation and poorly fitted and maintained pack saddles (11–13), together with the lack of a grooming practice. Wounds relating to tethering were identified primarily at the level of the pastern but also the cannon bone (Figure 8). Such injuries are common place across Morocco (11, 14, 73) and result from tethering and hobbling practices, which should be carefully distinguished and can easily be prevented (14, 75, 76).

All mules and both donkeys in TO were worked in traditional bits, with one exception (Table 2). In this case, the owner had purchased a stainless steel bit from someone in Imlil. By contrast, in AR, 40.6% of mules used the modern bit supplied by SPANA at least part of the time. No mules were worked in head collars in either village. The reason for favouring the traditional bit was repeatedly given as the mule being difficult to work without the owner being able to control the mule through this device. Bitting injuries were more commonly seen and most common and severe where the mule was worked in a traditional bit (Figure 9). Injuries were identified to the bars of the mouth (Figure 10A), roof of the mouth, sublingual tissues and tongue (Figure 10B) as well as to the underside of the jaw. Injuries were due largely to the damage caused by a thin metallic bitting device being used to subdue, control and direct the mule with little or no awareness of the damage caused. Some injuries were the result of metal wire being used to repair the bridle and/or attach the bit to the bridle (Figure 10B).

Hoof pathologies and hoof care

All mule owners used traditional farriery methods with closed shoes applied to the hoof by the local blacksmith. Shoeing was undertaken at a similar frequency in both Douars (mean time since last shoeing was 14.3 days in AR and 14.7 in TO) (Figure 11). This meant that hooves were not subject to wear when working on local terrain. The shoeing involved the use of a traditional cleaving tool that cut a wedge of horn from the foot in a sweeping action from heel to toe prior to applying new shoes, but did not involve any balancing of the foot (see Figure 11). As a result, many mules presented with a long toe, short heel conformation, with toe dumping a common practice. The closed nature of the shoe, traps material in the frog and makes hoof care difficult. There was no awareness of hoof hygiene – feet were not picked out and cleaned and mules were typically bedded in their own manure (Figure 12) because this was believed to keep the stable warm and because there is no litter (e.g., straw) available. During the winter months, mules are typically confined to their stables and their feet are therefore immersed in manure and urine. When manure was used as a bedding substrate, mules were significantly more likely to have an unhealthy hoof sole ($\chi^2 = 14.29$, $p = 0.0064$), with foot rot the most common finding (12 mules) leading to degeneration of the frog (Table 3, Figure 13). This is exacerbated by contracted heels and excessive trimming of the frog (77, p. 379). These factors mean that the hoof does not have opportunities to find its own natural healthy balance (78) and become resistant to abrasion and other insults. Instead, the sole has a tendency to become soft and vulnerable and owners have little confidence in the hoof's natural defences, believing that the heel needs to be kept covered and protected.

Concluding discussion

This paper has traced the various ways that local communities on the Toubkal have come to interact with the outside world, acquire and start working mules. Initially, mules were only owned by a few individuals. This gradually changed as opportunities emerged to earn money that rendered mules affordable and serviceable. With the advent of mountain tourism, the mule population in Aremd has grown substantially and now most families have a mule who is utilised to generate income wherever opportunities arise to do so. These developments have not been accompanied by improvements in mule care and awareness of both mulemanship and the complex network of actors contributing to mule welfare issues is limited (10, 11, 13, 79).

Each of the welfare issues reported here – poor body condition, pack wounds, tethering injuries, bitting injuries, and foot pathology are the product of a constellation of relational (10) socio-cultural, historical, geographical, technical, material, educational, and

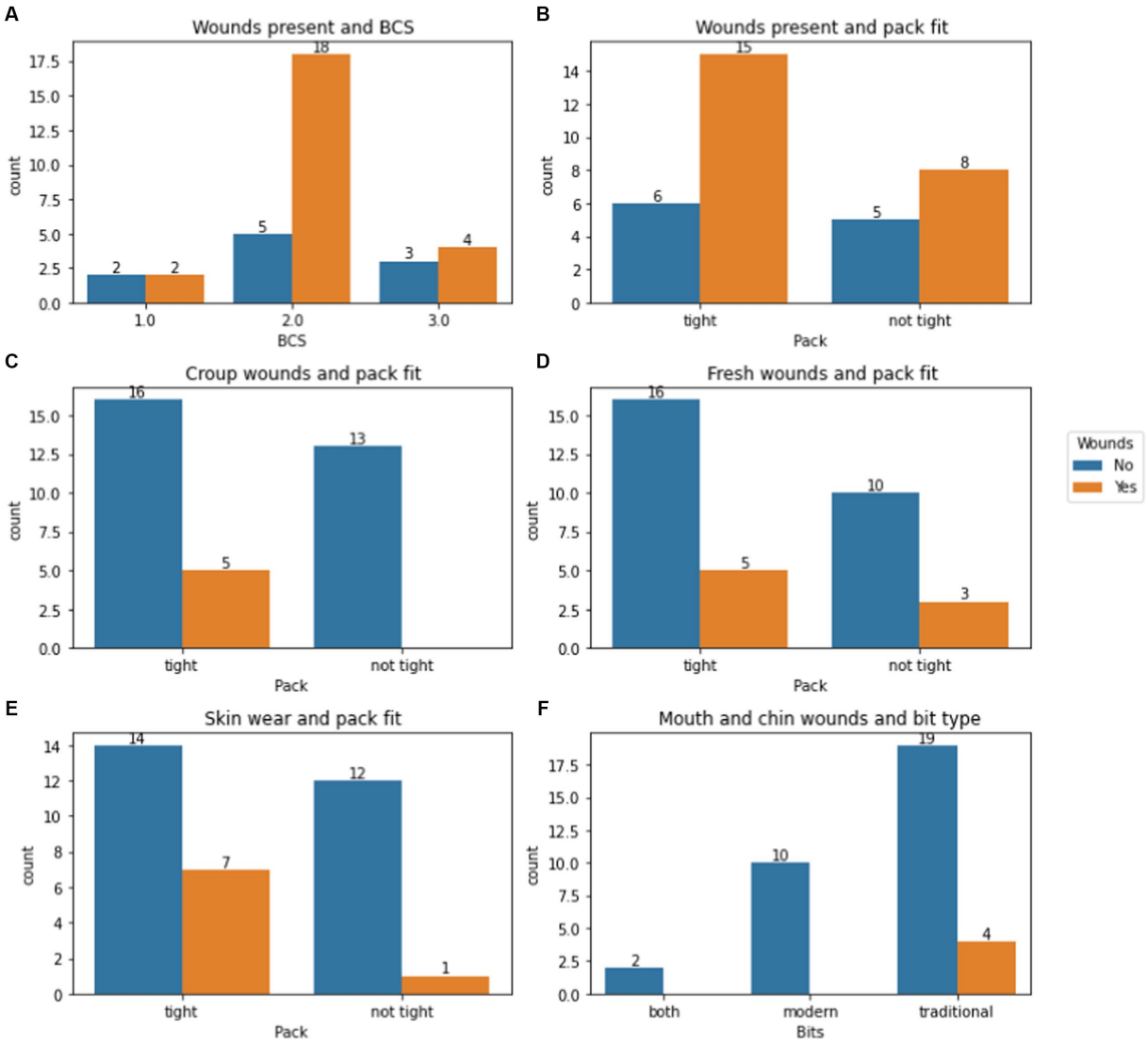


FIGURE 9
Bar charts illustrating number of mules with: **(A)** old or fresh wounds present, by BCS (*n* TO = 5; AR = 29); **(B)** old or fresh wounds present, by pack fit (*n* TO = 3; AR = 31); **(C)** croup wounds present, by pack fit (*n* TO = 3; AR = 31); **(D)** fresh wounds present, by pack fit (*n* TO = 3; AR = 31); **(E)** presence of wear to the skin, by pack fit (*n* TO = 3; AR = 31); and **(F)** mouth or chin wounds present, by bitting type (*n* TO = 3; AR = 31). The number of mules in each group is given above each bar.

economic factors (80). A systems approach to such problematics requires us to recognise the need to see beyond the presenting surface phenomena to the deeper elements of an iceberg model: the patterns of behaviour, the ways parts are related, structure the system and influence the patterns and the underlying mental models, as well as the values and beliefs that shape the system (80). We also need to recognise that the transmission of knowledge is both vertical between members of different generations, stereotypically between parents and their offspring but also horizontal between the members of the same generation (17, p. 58). This further requires us to recognise the complexity of such phenomena and adopt a collaborative and participatory approach (13, 14, 81) that moves beyond knowledge transfer (82) and into awareness-based systems change, recognising the need to address attitudes, mind sets, beliefs and mental models through cycles of facilitated reflection and action (10, 13, 31, 80, 81) as well as the more obvious practicalities that traditional veterinary health care interventions focus on. In the case of the system within and from which mule welfare arises, there needs to be recognition of the shared responsibilities of the stakeholders who, as a network, co-create mule welfare. The mining and mountain tourism industries have a long history of hiding human and animal welfare abuses underground and in other ways (6, 83–87). These include the abuses that lie hidden in the mouths (10) and under the pack saddles (13) of working equines as well as the abuses these animals are subject to when they are sold on to work in the cities. These issues are challenging, especially given the limited resources available to transform the system (see Figure 13).

A co-creative, collaborative approach seeks to realise the collective intelligence of the system (88, 89) and this means creating holding

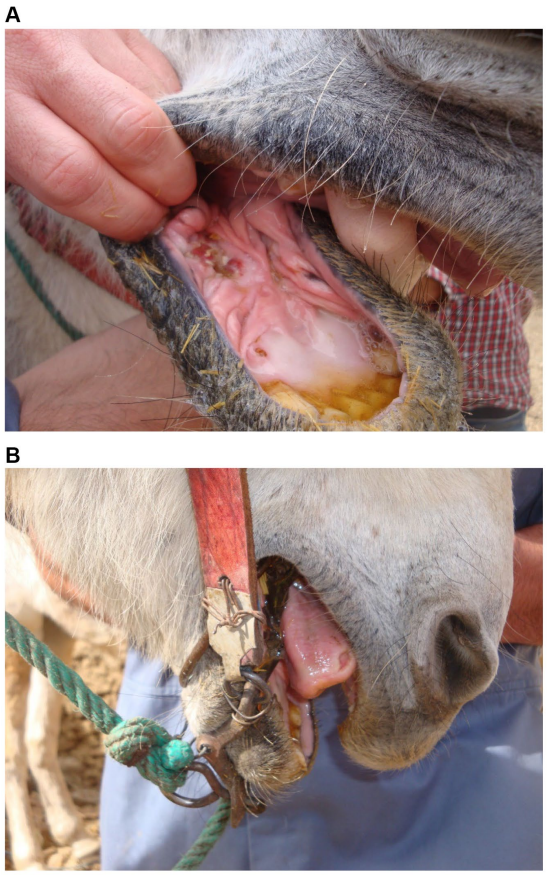


FIGURE 10
(A) and **(B)** The grey mule pictured here, from Tizi Oussem, shows injuries to both the bars of the mouth and tongue. The image below shows the use of metal wire to secure the leather bridle to the bit.

spaces for change to emerge (31) with those who are most open to delivering these emergent futures (77, 90) being supported to prototype new ways of relating and working with pack mules that transcend the disinterest, lack of compassion and fear-based absencing (10) that characterises current relations. Whilst swapping traditional bits out for modern stainless bits may be helpful (91) in addressing the worst of the injuries, it is important to recognise that these technical answers do not address underlying root causes. A systems approach (92–94) informed by detailed ethnographic understanding of relational working practices recognises that the relationships between many mules and their handlers are fear based and that training is required to facilitate a shift from domination to partnership (10, 95). As part of this it is helpful to recognise that head collars are more appropriate for ground work where the mule is unridden (10, 96). Owners can be taught basic shaping behaviours using positive reinforcement techniques that over time will allow mules to be handled easily and safely (31, 97). This takes times but is especially important as regular exposure to stress-free handling and to cleaning of the feet habituates mules to such interactions. Daily grooming and foot care also allow rubs, wounds and other health problems to be identified early.

Tourists themselves have a role to play by exercising their discretion in choosing service providers and by taking an active interest in the welfare of the mule who they travel with. Tourists are also increasingly reporting their concerns (90) in ways that demonstrate the importance of working equid welfare in tourism (7). Trekking agencies are able to write welfare specifications into their contracts with local agencies (13) and can develop and subscribe to industry codes of practice (96). Local authorities can introduce local regulations and NGOs such as SPANA can undertake technical inspections through their monthly visits to the area (91) and contribute to training initiatives, especially of local farriers, saddle makers and other artisans. Local muleteer associations have a crucial role to play in advocating for their members and professionalising the services they provide so that these revenues can be invested in better equipment, training and education. A key focus here, arguably needs to be on co-creating futures that are affordable and accessible whilst respecting mule welfare and the needs of the community. Such participatory and collaborative approaches are radical (97) and require significant capacity building and an investment in meeting and listening together. There is a clear role for research in this sector. Hossaini-Hilali reports that, between 1976–2011, only 28 scientific publications on equines were produced by students at the Institute Agronomique et Vétérinaire, from the 149 veterinary theses written during this time (98, p. 28). A shift in the kind of research

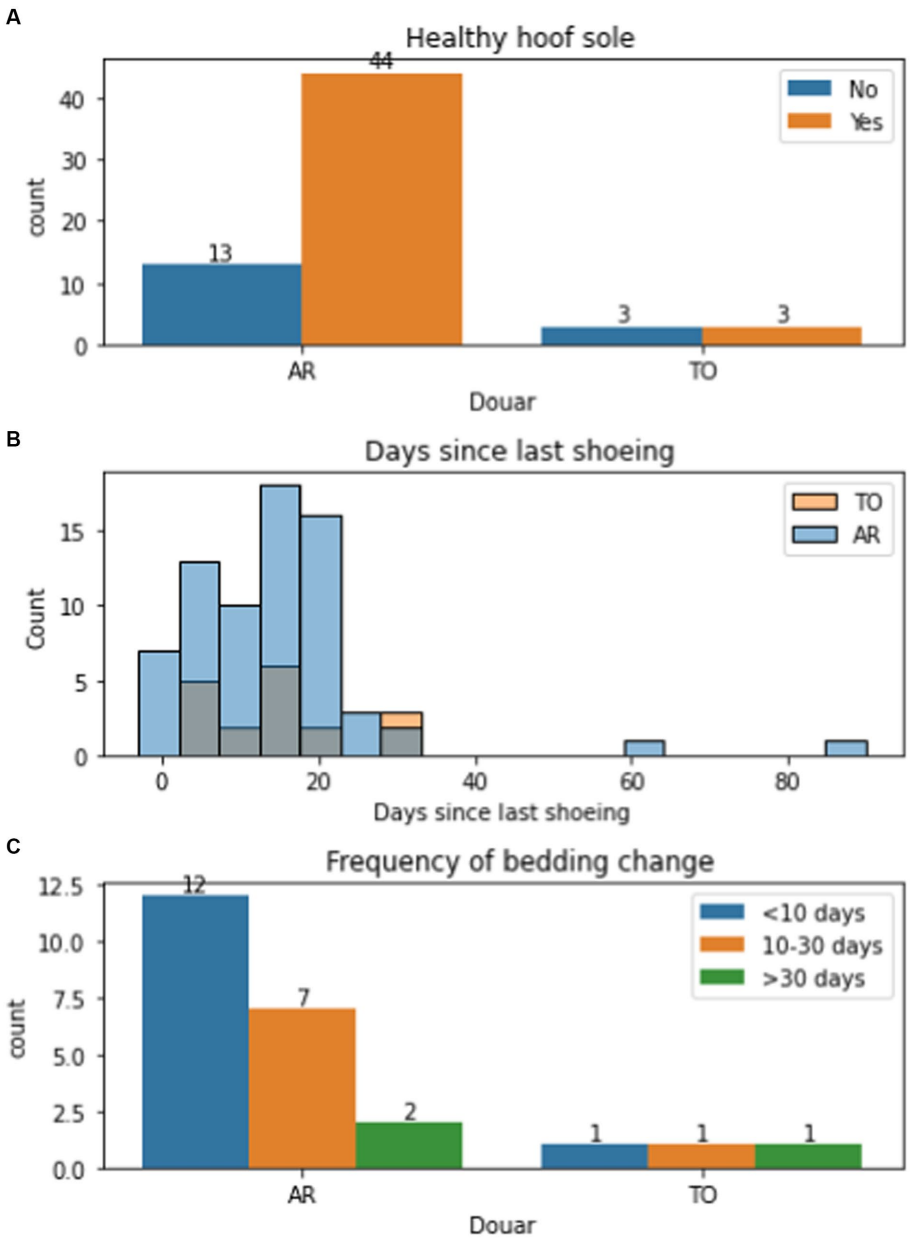


FIGURE 11
(A) Presence of healthy foot sole by Douar (village; Aremd (AR) and Tizi Oussem (TO)), with number per group given above the bar; **(B)** layered histogram of days since last shoeing in AR and TO; and **(C)** frequency of bedding change in AR and TO, with number per group given above the bar.

undertaken is also needed if the inter-relationships between agriculture, mule welfare and tourism are to be unpacked. Allali, in his call for sustainable tourism, highlights the opportunity provided by seeing the impact of tourism on the local environment that arises when people work both in agriculture and tourism (99, p. 69). The reduction in the amount of cereal production arising from decisions to plant trees (19), with these occupying 80% of the agricultural area surveyed (100, p. 69) similarly shows how decisions in one sphere have impacts elsewhere – specifically on the food available to livestock. A study, similar to that reported recently from Kashmir (101) is thus needed to determine the volume and nutritional quality of the grain and fodder currently eaten by local mules and how the system can better provide for the nutritional needs of working pack mules. This could include payments in kind that go directly to the mule and that subvert exploitative practices.

In conclusion, there is a need for more collaborative partnerships across the mountain tourism system to allow communities and their mules to break free from the constraints of the limited means and rationalities, the narrow valleys, mind-sets and relational practices that have historically limited their ability to realise more optimal welfare.

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FIGURE 12
Mules are stabled overnight and through the winter in ground level stables. The manure is allowed to build up and is believed to keep the stable warm. Mules are typically tethered by the foot and not free to move around, often because owners find it easier to catch them this way. This reflects the lack of connection, mutual trust and respect between the handler and their mule.

TABLE 3 **Healthy hoof sole and associated management factors in mules.**

		Healthy hoof sole	Unhealthy hoof sole
Bedding state	Dry	15	5
	Wet	1	8
Manure used as bedding	Yes	5	12
	No	15	1
Frequency of bedding change	<10 days	9	3
	10–30 days	2	5
	>30 days	2	1
Frequency of mucking out	Daily or less	2	0
	2–10 days	6	0
	10–15 days	2	4
	>15 days	6	7

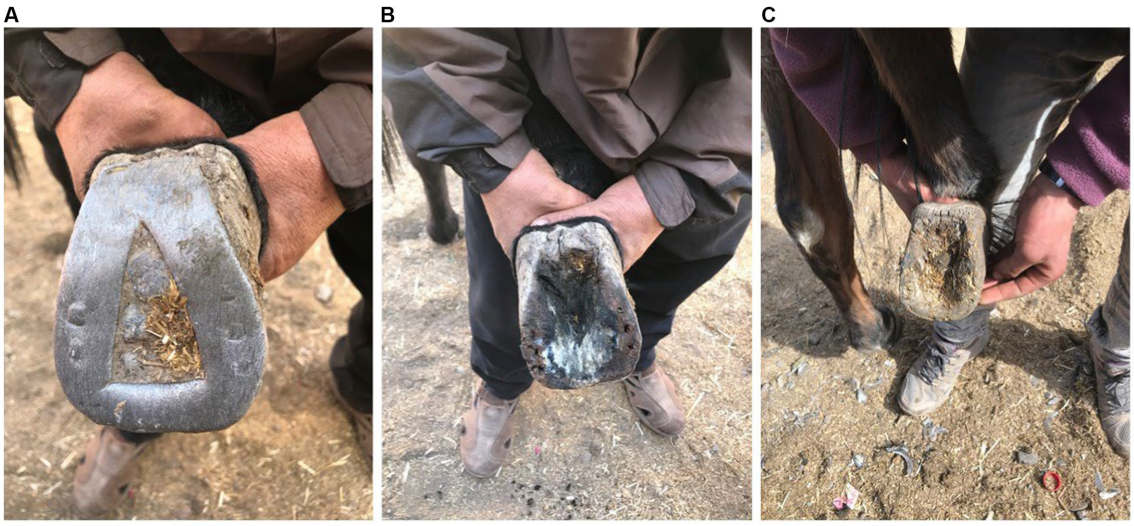

FIGURE 13
The traditional closed shoe is bent over the heel and does not allow healthy functioning of the frog **(A)**. It allows soiled bedding and other material to become trapped around the frog leading to maceration of the sole **(B)** and degeneration of the frog (**B,C**).

Data availability statement

The datasets presented in this article are not readily available because the animal data collected has been provided by SPANA Maroc. Requests to access the datasets should be directed to GC glen.cousquer@ed.ac.uk.

Ethics statement

The ethnographic part of this study is reported retrospectively here. The original study was approved by the Ethical Committee of the University of Edinburgh's School of Geosciences. The animal studies were approved by R(D)SVS Veterinary Ethics Research Committee. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was not obtained from the owners for the participation of their animals in this study because oral consent was deemed more appropriate given the cultural contexts in which the work was undertaken. Written informed consent was not obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article because written consent is not deemed culturally appropriate and oral consent was used instead.

Author contributions

GC: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing. HA: Resources, Supervision, Writing – review & editing. VL-M: Formal analysis, Software, Writing – review & editing.

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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.

Publisher's note

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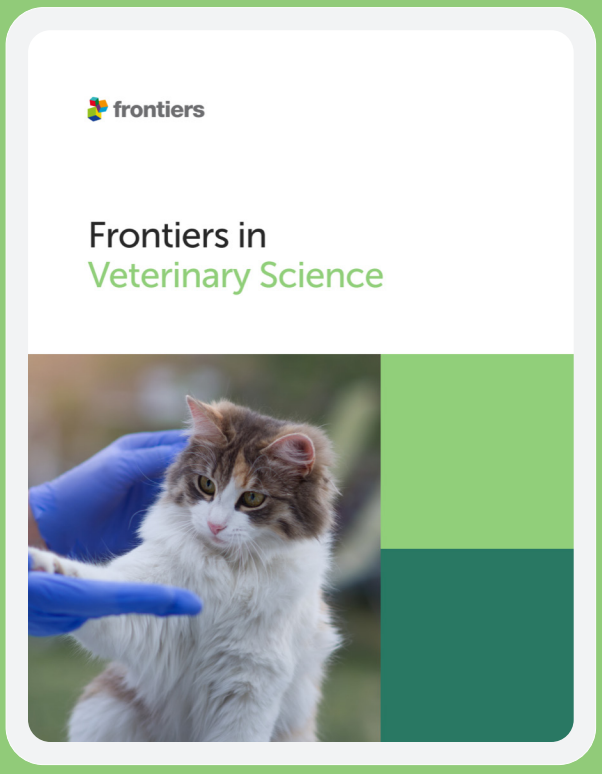

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