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Epidemiological Study on Caprine Babesiosis: Insights from Indigenous Goat Breeds Treatment Efficacy and Management

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AN, SKA and RA conceived the idea and executed the study. MK, SEUH and AS performed PCR study and analysis, MI, AN and AAS helped in the manuscript write up. All the authors proofread the manuscript before the final submission.

Key words
***Babesia ovis*, Beetal goat, Imidocarb Teddy goat, Tick borne disease**

ABSTRACT

Caprine babesiosis, a disease of significant economic concern, was studied in three prominent goat breeds beetal, teddy, and crossbred (Beetal × Teddy) in the Jhang district of Punjab, Pakistan. Blood samples were randomly collected from 300 goats to assess the prevalence of *Babesia ovis*, evaluate hematological parameters of infected goats before and after treatment, and determine the efficacy of a therapeutic regimen combining Imidocarb dipropionate and oxytetracycline. Additional data on breed, age, sex, parity, floor type, flock size, feeding system and flock composition were gathered through a questionnaire. *B. ovis* prevalence was determined using both microscopy of Giemsa-stained thin blood smears and polymerase chain reaction. Of the 300 goats tested, 23.6% (95% CI ± 4.8%) were microscopically positive for *B. ovis*, while PCR revealed a slightly higher prevalence of 28.2% (95% CI ± 4.8%), with no statistically significant difference ($P > 0.05$) between the two methods. Males exhibited a non-significantly higher infection rate compared to females, while goats aged >6 months to <1 year showed a significantly higher infection rate ($P < 0.05$). Infection rates in goats reared under intensive feeding systems, medium-sized herds, and mixed-species flocks demonstrated significantly higher infection prevalence. Significant post-treatment improvements in erythrocyte count, hemoglobin levels, and hematocrit were found by hematological tests. Imidocarb dipropionate and oxytetracycline worked very well together, indicating that this treatment plan can effectively cure caprine babesiosis in Pakistan.

INTRODUCTION

Babesiosis is a critical hemoparasitic disease affecting various domestic animals, including sheep, goats, buffalo, and cattle. It is caused by Babesia protozoan parasites, which are mainly spread by ixodid ticks. The disease presents a wide array of clinical symptoms, such as fever, anemia, hemoglobinuria, and icterus, all of which contribute to the debilitation of affected animals and significant economic losses (Schnittger *et al*., 2004; Aktas *et al*., 2007). In goats, three major species of *Babesia* are responsible for the infection: *B. motasi*, *B. ovis*, and *B. crassa*. Among these, *B. ovis* is the most virulent, with mortality rates ranging from 30% to 50% in both sheep and goats if left untreated (Shahzad *et al*., 2013). The economic implications of babesiosis extend beyond direct animal mortality. The disease negatively affects livestock productivity by causing reductions in milk, meat, and wool

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yields, as well as impairing reproductive performance. Additionally, the presence of babesiosis in a herd poses risks to international trade, as infected and asymptomatic animals can serve as reservoirs for disease transmission (Nagore *et al*., 2004). This risk of disease spread is particularly concerning for nations that rely heavily on livestock farming for economic stability. As a result, controlling babesiosis is critical not only for safeguarding animal health but also for maintaining the economic viability of farming operations and livestock-related trade.

Globally, caprine babesiosis has been documented in various regions through both microscopy and molecular techniques like polymerase chain reaction (PCR). Studies in Iran, for example, have reported a prevalence rate of 6.8% in the Khorasan region and 12.2% in West Azerbaijan, underscoring the widespread distribution of the disease (Razmi *et al*., 2003; Esmaeilnejad *et al*., 2014). Three main tick borne diseases, theileriosis, anaplasmosis and babesiosis are reported to affect small ruminants and cause huge economic losses in Pakistan (Jabbar *et al*., 2015). The prevalence of caprine babesiosis in small ruminants investigated in the Southern Punjab region of Pakistan lies between 7-58.5% in sheep and 7-23% in goats (Iqbal *et al*., 2011; Khan *et al*., 2022). These prevalence rates, although variable, suggest that babesiosis is a persistent and ongoing threat to goat populations, particularly in countries where tick infestation is prevalent. The diagnostic approach to babesiosis depends on the stage of the infection. At the clinical level, the disease can be diagnosed through observable symptoms such as fever, lethargy, and jaundice, alongside microscopic examination of blood smears for the presence of *Babesia* parasites (Schnittger *et al*., 2003). However, subclinical infections, where symptoms may not be immediately apparent, are best detected using more sensitive diagnostic methods such as PCR and serological assays (Modi *et al*., 2015; Farooq *et al*., 2020). These assays allow for early and accurate detection of the pathogen, thereby enabling more effective control and treatment strategies. Treatment of babesiosis in goats typically involves the use of antiprotozoal drugs such as Diminazine aceturate and Imidocarb dipropionate being the most commonly administered compounds. Oxytetracycline is often used in combination with these drugs to enhance efficacy (Ijaz *et al*., 2013). The effectiveness of treatment regimens varies by region, with reports indicating a success rate of 60-100% depending on the local conditions and the drug formulations used (Ijaz *et al*., 2013; Anonymous, 2020). Despite the availability of these treatments, the variable efficacy of the drugs highlights the need for region-specific studies to evaluate the optimal therapeutic protocols for controlling babesiosis.

In Pakistan, where livestock farming is a cornerstone of the rural economy, goats play a vital role in supporting the livelihoods of millions of people. According to recent estimates, the goat population in the country stands at approximately 78.2 million (Modi *et al*., 2015; Anonymous, 2020), underscoring their significance in the agricultural sector. Given this large population and the economic importance of goats, the control of diseases like babesiosis is crucial. Infected animals not only suffer from the disease but also pose a risk to the overall productivity of the livestock sector. The present study was undertaken to assess the prevalence of *Babesia* spp. infections in goats in the Jhang district of Punjab, Pakistan, using both microscopy and molecular detection methods. In addition, this study aimed to evaluate the efficacy of locally available drug formulations containing Imidocarb dipropionate for treating babesiosis. By combining clinical observations with advanced diagnostic techniques, this research seeks to provide valuable insights into the control of babesiosis in Pakistan's goat population, thereby contributing to improved livestock health and economic stability.

MATERIALS AND METHODS

Study animal

The study involved three commonly raised local goat breeds namely beetal, teddy, and crossbred (Beetal × Teddy), representing a range of ages, both sexes, and varying parities within small ruminant flocks in the Jhang district of Punjab, Pakistan (Fig. 1). This diverse sampling allowed for a comprehensive analysis of caprine babesiosis across different demographic and management conditions. Data pertaining to potential risk factors such as breed, age, sex, parity, floor type, flock size and flock composition were gathered through a predesigned questionnaire at the time of sampling and their association with the prevalence of the diseases was evaluated.

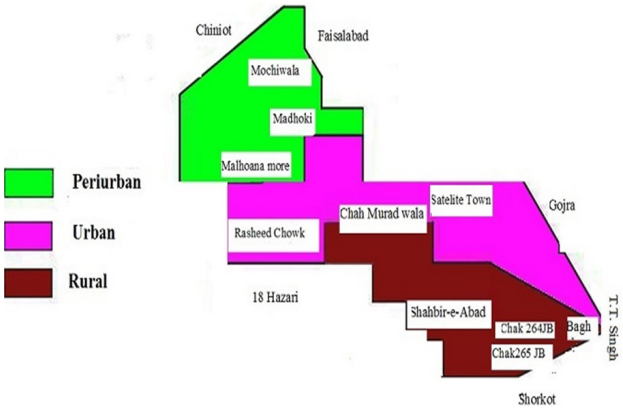

Fig. 1. Map of study areas showing sampling sites of Peri-urban, urban and rural area in Tehsil Jhang, Punjab, Pakistan.

Microscopic examination

Blood samples (5 ml each) were randomly collected from beetal (n=100), teddy (n=100), and crossbred (Beetal × Teddy, n=100) goats, raised in 10 flocks within the study area. These flocks included both mixed species (sheep and goats) and goat-only herds. Sampling was conducted between May and August. Blood was aseptically drawn from the jugular vein using a 10 ml disposable syringe and immediately transferred into labeled vacutainers containing the anticoagulant EDTA.

Blood smear preparation and Giemsa staining was done according to our standard lab procedures sample was placed on a clean and dry (grease-free) glass slide near an end. Another dry slide with a plane edge was placed near the blood drop on the first glass slide at a 45º angle and spread it uniformly. After drying of the slides were soaked in absolute methyl alcohol for 3-5 min and the slides were stained with 10% diluted Giemsa stain and observed under oil emersion lens (100X magnification) in the laboratory.

Polymerase chain reaction (PCR) examination

After microscopic examination, 24 randomly selected positive samples and 15 negative samples were further tested using PCR for confirmation. DNA was extracted manually, and the PCR assay was performed to detect *B. ovis* based on the protocol described by Esmaeilnejad *et al*. (2014). A specific pair of primers, BABF (5′-GCGATGGCCCATTCAAGTTT-3′) and BABR (5′-CGCCTGCTGCCTTCCTTAGA-3′), was used to amplify a 146 bp fragment of the *18S rRNA* gene of *B. ovis* (Theodoropoulos *et al*., 2006).

The 5 µl of genomic DNA, 25 µl of master mix, 2 µl of forward primer, 2 µl of reverse primer, and 16 µl of nuclease-free water were used in the 50 µl PCR process. After a three-minute initial denaturation phase at 94°C, there were 35 cycles of denaturation at 94°C for 45 sec, annealing at 56°C for 45 sec, and extension at 72°C for one minute. Ten min of a final extension were conducted at 72°C. Following a 1.5% agarose gel electrophoresis analysis of the PCR results, bands were observed using a UV trans-illuminator.

Hematological examination

Hematological analysis was performed using the Exigo Veterinary Hematology Analyzer (Boule Medical, Sweden) as described by Ijaz *et al*. (2013). The blood samples (n=62) were collected from the animals used in the treatment trial to evaluate the impact of *Babesia* infection on several key hematological parameters, comprising white blood cell (WBC), erythrocyte (RBC) counts, packed cell volume (PCV), hemoglobin (Hb) levels, lymphocytes, monocytes and neutrophils. These indicators provided insight into the extent of anemia and immune response in the infected goats, helping to better understand the hematological alterations associated with caprine babesiosis.

Treatment strategy

A total of 62 *Babesia* positive goats comprising 12 beetal goats were treated with Imizol (Imidocarb dipropionate, ICI, Pakistan) intramuscularly @ 1 ml/100 kg body weight (BW), alongside oxytetracycline (Oxy-5® Injection 50mg/ml, Selmore Pharmaceutical Pvt. Ltd. Pakistan) @ 10 mg/kg BW. While, 25 Teddy goats were treated with Redmido (Imidocarb dipropionate, Leads Pharma, Pakistan) at the same dosage of 1 ml/100 kg BW, combined with oxytetracycline @ 10 mg/kg BW. Similarly, 25 crossbred goats (Beetal × Teddy) were treated with Imipro (Imidocarb dipropionate, Selmore Pharmaceuticals, Pakistan) @ 1 ml/100 kg BW, also in combination with oxytetracycline administered intramuscularly @ 10 mg/kg BW. Seven days post-treatment, the previously positive animals were re-examined using Giemsa-stained thin blood smears to assess the efficacy of the therapeutic regimens. Additionally, the effect of the treatment on hematological parameters was evaluated using a fully automated Exigo Veterinary Hematology Analyzer (Boule Medical, Sweden). This follow-up examination provided insights into the drugs' effectiveness and their impact on improving the hematological indices in the infected goats.

Statistical analysis

The efficacy of the treatment was calculated using the following formula:

$$\text{Efficacy} = \frac{\text{Number of recovered animals}}{\text{Number of infected animals}} \times 100$$

A paired sample t-test was employed to compare the pre- and post-treatment hematological parameters. Additionally, the Pearson chi-square test was used to analyze prevalence rates, while the likelihood ratio test was applied to evaluate the therapeutic efficacy of the drugs. All statistical analyses were conducted using SPSS software, version 16.

RESULTS

The overall prevalence of babesiosis based on microscopy was 23.6% (± 4.8%, 95% CI), while the PCR assay indicated a slightly higher, though statistically non-significant ($P > 0.05$), prevalence of *B. ovis* at 28.2% (± 14.1%) among beetal, teddy, and crossbred goats (Fig. 2A). In terms of age, the incidence of *Babesia* infection was significantly higher ($P < 0.05$) among goats aged 6 months to 1 year, with a prevalence of 35.0% (± 16.8%).

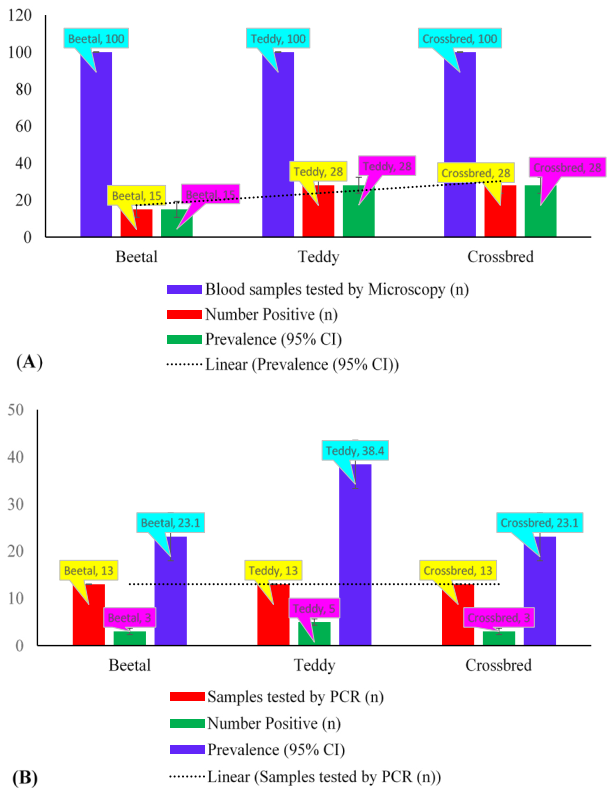

Fig. 2. Prevalence of babesiosis in goats from Jhang Tehsil using microscopy (A) and PCR assay (B).

In contrast, the lowest incidence (15.5% ± 10.5%) was observed in goats aged 4 to 7 years, indicating a possible age-related susceptibility to infection (Fig. 2B).Sex and parity were not found to be significant factors influencing disease prevalence. Although *B. ovis* infection tended to be higher in primiparous goats (25.0% ± 21.0%) compared to second (16.6% ± 11.1%) and third parity goats (13.0% ± 13.7%), this difference was not statistically significant ($P > 0.05$). The prevalence of *B. ovis* was significantly ($P<0.05$) higher in goats kept in medium-sized goat flocks (15-30 goats) than in small ones (1-15 goats). Likewise, the prevalence of *B. ovis* infection was significantly ($P<0.05$) higher in flocks rearing sheep and goats together, than in those flocks having goats only (Table I). The hematological parameters showed that the mean values of RBCs, HB, and HCT seven days post-treatment, were significantly ($P<0.05$) higher than measured pre-treatment, while WBCs didn't differ significantly from the known standard values (Table II). Imizol (ICI Pvt. Ltd. Pakistan), Redmido (Leads Pharma Pvt. Ltd. Pakistan), and Imipro (Selmore Pharmaceutical Pvt. Ltd. Pakistan), when used in combination with oxytetracycline (Oxy-5® Injection 50mg/ml, Selmore Pharmaceutical Pvt. Ltd. Pakistan), were found to be 100% (± 0%), 88.0% (± 12.7%), and 80.0% (± 15.9%) effective, respectively, although the differences were not statistically significant ($P > 0.05$). A total of 62 goats were treated with one of the three treatment regimens, resulting in an overall efficacy of imidocarb dipropionate combined with oxytetracycline at 87.1% (± 8.3%) (54/62, at 95% CI) among the treated animals. This demonstrates the generally high effectiveness of imidocarb dipropionate-based treatment, despite some variation across formulations (Table III).

Table I. Various risk factors associated with caprine babesiosis.

Level	No. of animals tested	Positive	Prevalence %
Age			
0-6 months	42	11	26.2^{a}±13.2
6 months-1 year	31	11	35.4^{b}±16.8
>1-2 years	75	18	24^{c}±9.6
>2-3 years	68	15	22^{d}±9.8
>3-4years	39	9	23.2^{e}±13.2
>3-4years	45	7	15.5^{f}±10.5
>4-7 years	42	11	23.6±4.8
Sex			
Male	60	17	28.3^{a} ± 11.5
Female	240	54	22.5^{b} ± 5.2
Parity			
First parity	16	4	25^{a} ±21
Second parity	42	7	16.6^{b} ±11.1
Third parity	23	3	13.0^{c} ±13.7
Floor type			
Dirt yard	195	90	46.15^{a}±6.9
Paved	91	32	35.16 ±9.8
Cement	14	4	28.57 ^{b} ±23.6
Flock size			
1-15	120	28	23.33^{a}±7.5
15-30	180	112	62.22 ^{b} ±7.0
Feeding system			
Pasture grazing	125	27	21.6^{a}±7.2
Semi-intensive	82	6	7.3^{b}±5.6
Stall fed/ intensive	93	38	40.8^{c}±10.0
Pasture grazing	125	27	21.6^{a}±7.2
Flock composition			
Goat only	41	11	26.82 ^{c} ±13.5
Sheep and goats	259	117	45.18 ^{d} ±6.0

*Values with different alphabets in superscript show statistically significant difference ($p<0.05$) associated with various risk factors.

Table II. Effect of babesiosis on hematological values (Mean±SD) of beetal, teddy, and crossbred goats before and after treatment for babesiosis.

Hematological parameters	Beetal goats		Teddy goats		Crossbred goats	
	Before treatment (D 0)	After treatment (D 7)	Before treatment (D 0)	After treatment (D 7)	Before treatment (D 0)	After treatment (D 7)
WBCs (10^3/µl)	9.09 ± 2.69	8.63 ± 3.01	6.77± 4.01	4.98 ± 2.17	7.07 ± 2.76	8.17 ± 3.83
RBCs (10^6/µl)	5.22 ± 0.43 [a]	5.88± 0.90[b]	5.40± 1.33[a]	6.09± 0.80[b]	4.90± 0.84[a]	5.95± 0.68 [b]
Hb (g/dl)	5.04± 0.83[a]	5.66± 1.44 [b]	6.03± 1.15[a]	6.82± 1.14[b]	5.61± 0.98	6.25± 1.00
HCT	29.85± 39.44[a]	254.85±32.7[b]	20.6± 3.36[a]	30.5± 12.2 [b]	46.59± 32.26[a]	63.01± 31.6[b]
Lymphocytes	6.30 ± 2.44	7.61± 1.61	5.45 ±1.52	6.34±2.31	4.82 ± 1.28	5.56 ± 2.35
Monocytes	6.29 ± 1.62	6.38± 281	1.20 ±0.58	1.58±0.93	1.20 ±0.58	1.58±0.93
Neutrophils	14.03 ± 9.29	8.98± 8.01	5.18 ±6.68	6.04±5.21	2.28 ±1.00	1.83±1.50

Values with different superscripts in alphabets in the same column and with (P<0.05) are significantly different, Paired sample T- test.

Table III. Efficacy of Imidocarb containing commercial products at 1ml/100kg BW i.m., administered in combination with oxytetracycline at 10 mg Kg^{-1} for treating caprine babesiosis.

Breed	Drugs	Treated (n)	Recovered (n)	Efficacy (%) ± (95%CI)
Beetal	Imizol®	12	12	100.0*±0.0
Teddy	Redmido®	25	22	88.0*±12.7
Crossbred	Imipro®	25	20	80.0*±15.6
Total		62	54	87.1±8.3%

*Indicates non-significant difference (P < 0.05) using Likelihood ratio.

DISCUSSION

The present study investigated the prevalence of *B. ovis* in goats using microscopy with Giemsa-stained thin blood smears, revealing an overall prevalence of 23.6% (±4.8%). In contrast, PCR analysis indicated a higher prevalence of 28.2% (±4.8%), likely due to the enhanced sensitivity of this method. These findings are in agreement with previous research, which reported a 33% prevalence of *B. ovis* in small ruminants across five districts in Southern Punjab, Pakistan (Iqbal *et al*., 2013). Both our study and the 2013 report originate from regions characterized by similar geographical and climatic conditions, which may contribute to the comparable prevalence rates observed. Our results indicated a significantly higher prevalence (35.4% ± 16.8%) of *Babesia* infection in younger goats aged 6 months to 1 year. This finding aligns with earlier studies and can likely be attributed to their lower immunity to babesiosis, as maternally transferred immunity typically diminishes by this age. Additionally, we noted a lower prevalence (26.2% ± 13.2%) in goat kids up to 6 months, a period during which maternally derived antibodies persist (Czopowicz *et al*., 2018). While the prevalence of *B. ovis* tended to be higher in male goats, this difference was not statistically significant. This finding is consistent with the research conducted by Iqbal *et al*. (2013), suggesting that the likelihood of tick infestations remains similar across both sexes and various parities throughout the goats' lifespan.

In our study, the semi-intensive system of goat rearing was associated with higher tick exposure, potentially due to the influx of ticks from pastures, grasslands, and forests where other livestock species, including cattle and wild ruminants, may graze. Moreover, close confinement during partial stall feeding could contribute to increased tick acquisition from neighboring animals. We observed a significantly higher incidence of *B. ovis* in medium-sized flocks (15-30 goats) and in flocks that included both sheep and goats compared to smaller, single-species goat flocks. This is consistent with the findings of Iqbal *et al*. (2013), suggesting that the lack of separate enclosures for different species may facilitate transmission between them. Additionally, sheep, being woolly animal, may act as reservoir for ticks, promoting interspecies transmission in close quarters. This outcome parallels another study reporting significantly higher tick infestation rates in Tellicherry goats (78.2%) and Madras red sheep (77.1%) in Tamil Nadu, India (Soundararajan *et al*., 2018).

Our findings are also in line with those of Sevinc *et al*. (2007), who reported decreased values for RBCs, Hb and PCV, alongside increased WBC counts in infected animals. Similar trends were observed in other studies involving sheep and goats (Esmaeilnejad *et al*., 2012). The reduction in RBC counts can be attributed to the rapid destruction of these cells caused by the intra-erythrocytic presence of protozoa. Notably, the hematological profile of recovered animals showed significant improvement

in these parameters following treatment. In terms of treatment efficacy, Imizol combined with oxytetracycline demonstrated 100% efficacy in beetal goats, corroborating the findings of Ijaz *et al*. (2013), which reported a 97.3% efficacy of Imidocarb in sheep and goats. Redmido, when combined with oxytetracycline, yielded an efficacy of 88.0% (± 12.7%), which is consistent with the results of Rashid *et al*. (2010), though inconsistent with Ijaz *et al*. (2013). In contrast, Imipro with oxytetracycline was found to be less effective, a finding that aligns with Ajith *et al*. (2017), and may be attributed to variations in manufacturing quality and composition compared to other tested drugs. Despite the minor differences in efficacy among the formulations, all demonstrated comparable effectiveness in treating caprine babesiosis. The overall combined efficacy of the three regimens containing imidocarb dipropionate and oxytetracycline was 87.1%, supporting the use of imidocarb dipropionate as a therapeutic option against caprine babesiosis. Therefore, all three commercial products are viable treatment options, with Imizol® (ICI, Pvt. Ltd., Pakistan) emerging as the preferred choice for treating caprine babesiosis in field conditions.

CONCLUSION

This study offers valuable insights into the current prevalence of caprine babesiosis across different breeds of goats in Tehsil Jhang, Punjab, Pakistan, and aligns with findings from previous research. The results can inform veterinarians in developing more effective therapeutic strategies for managing caprine babesiosis. All three commercial formulations containing imidocarb dipropionate combined with oxytetracycline were shown to be effective against *B. ovis*. Additionally, the study highlights the importance of keeping sheep and goats in separate enclosures to reduce the risk of tick-borne disease transmission. Improving flooring conditions preferably paving can significantly minimize tick propagation and maintenance in small ruminant facilities. Furthermore, awareness campaigns aimed at educating farmers about the control and treatment of hemoprotozoan infections and tick infestations could prove highly beneficial for the health of small ruminants.

DECLARATIONS

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The experimental consumables were provided from the University resources.

IRB approval

Approved the University Institutional Ethical Committee.

Ethical approval

The Institutional Committee for the Ethical Use of Animals in Research and Teaching at the University of Veterinary and Animal Sciences, Lahore (Jhang Campus) granted ethical approval for the study (DAS/598, dated 20-07-2017), and the experiment was conducted in strict accordance with the committee's guidelines. Before the goats were sampled and treated, the owners gave their informed consent.

Generative AI and AI-assisted technology statement

The authors have declared that no generative AI or AI-assisted technologies were used to create this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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