


Impact of production practices and sanitary management on the prevalence of *Neospora caninum* and bluetongue virus in Creole goats from the tropical dry forest of Utcubamba, Peru

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OBJECTIVE

This study aimed to report the impact of production practices and sanitary management on the prevalence of *Neospora caninum* (*N caninum*) and bluetongue virus (BTV) in Creole goats from the tropical dry forest of Utcubamba, Peru.

METHODS

354 blood samples were obtained from male and female goats of diverse ages reared under an extensive productive system. The diagnosis of *N caninum* and BTV was conducted through a commercial ELISA kit (iD.vet) with readings taken on a Bio-Rad iMark microplate reader at 450 nm. Additionally, a survey was conducted with 18 goat producers to categorize them based on social, technical (production, health, reproduction, nutrition, and infrastructure), environmental, and economic factors.

RESULTS

The overall seroprevalence of *N caninum* and BTV in goats was 6.21% and 7.06%, respectively. Notably, group II farmers had the highest prevalence rates at 7.69% for *N caninum* and 23.08% for BTV. This group comprises producers with fewer than 21 goats, while group III includes 14.3% of producers with over 60 goats and better infrastructure.

CONCLUSIONS

This study confirms the presence of *N caninum* and BTV in goats of Utcubamba, Peru. Group II displayed the highest prevalence rates, suggesting that herd size and management infrastructure influence disease prevalence.

CLINICAL RELEVANCE

In the tropical dry forest of Utcubamba, inadequate management of *N caninum* carrier agents (eg, nonde-wormed dogs) heightens transmission risk, while the tropical climate supports BTV vectors. Effective diagnosis and health management strategies, including vector control and biosecurity practices, are crucial for ongoing herd health monitoring.

Keywords: goats, *Neospora caninum*, bluetongue, ELISA diagnosis, Amazonas

Goat farming is a crucial economic activity in Peru, particularly for low-income households. With an estimated population of around 2,000,000 goats,

producing over 5,000 tons of meat¹ and 18,000 tons of milk² annually, primarily utilized for artisanal cheese, this sector is vital to local economies. Most goat production systems in Peru are extensive.³ These systems are frequently affected by infectious and parasitic diseases, requiring stringent hygiene practices to mitigate disease transmission.⁴ The prevalence of such diseases negatively impacts productivity and economic returns.

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Neospora caninum and bluetongue are diseases of domestic ruminants that present significant challenges to public health, animal health, and livestock production worldwide. Neosporosis, caused by *N. caninum*, is a substantial contributor to abortion and reproductive disorders in ruminants.⁵ This disease is widely distributed, with goat infections often linked to factors such as age, sex, the presence of dogs, and reproductive issues on farms.⁶ In South America, the seroprevalence of neosporosis varies considerably, attributed to the nature of production systems and deficiencies in nutritional and sanitary management.⁷⁻⁹ Compared to other species such as cattle¹⁰⁻¹² and camelids,¹³ reports concerning neosporosis in goats in Peru are sparse.¹⁴

Bluetongue is a noncontagious viral disease affecting domestic and wild ruminants, including sheep, cattle, goats, buffaloes, antelopes, deer, elk, and camelids.¹⁵ Transmission occurs through the bites of infected mosquitoes from the *Culicoides* genus.¹⁶ The incidence of bluetongue is closely associated with the distribution of *Culicoides* mosquitoes, which is influenced by climatic factors.¹⁷ Clinical manifestations of the disease include fever, excessive salivation, oral mucosal ulcers, dyspnea, lameness, and lingual cyanosis.¹⁸ The disease leads to considerable economic losses in the livestock sector due to morbidity, mortality, reproductive failures, and decreased productivity.^{17,19} In goats, cattle, and

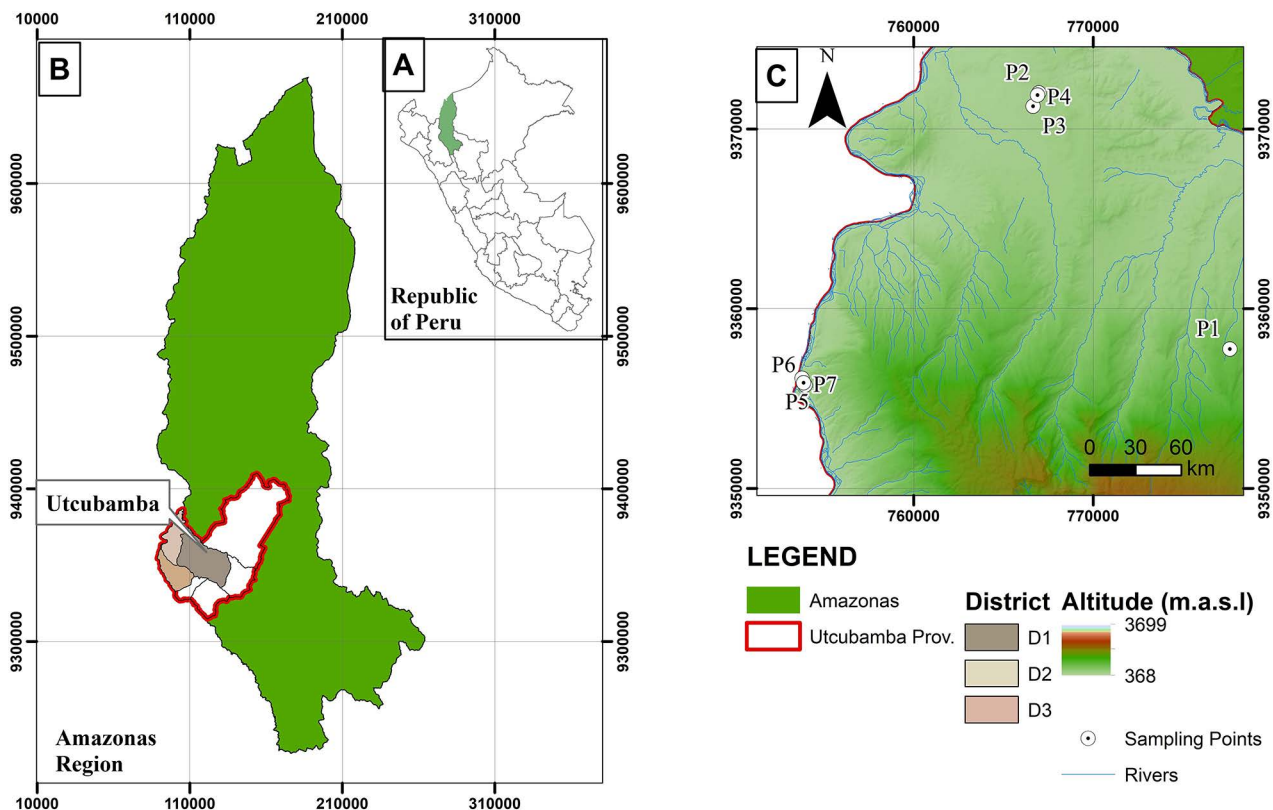
camelids, bluetongue can sometimes be asymptomatic.²⁰⁻²² In northern Peru, bluetongue has been reported in goats with a prevalence of 23.8%, showing a strong correlation with animal age,²³ and in the Peruvian jungle with a prevalence of 9.2%.²⁴

In the tropical dry forest region of Amazonas, Peru, goat farming faces challenges including inadequate health management, lack of effective diagnostic tools, and limited disease monitoring. Furthermore, there is a notable absence of data on the prevalence of *N. caninum*, and only 1 study²⁴ has investigated bluetongue in goats from this region, albeit it involved a small sample size. Consequently, this study aims to determine the prevalence of *N. caninum* and bluetongue virus (BTV) in Creole goats from the tropical dry forest of the Amazon region in Peru, considering producers' characteristics.

Methods

Study area

This study was conducted on 12 producers belonging to the Asociación de Productores Agropecuarios Residentes en el Sector La Cruz Baja Buena Vista from the Bagua Grande district and 6 independent producers from the districts of El Milagro and Cumba in the Utcubamba province of the Amazonas region (**Figure 1**). Sampling sites ranged in altitude from 458 meters above sea



level (El Milagro) to 1,026 meters above sea level (Bagua Grande). The Utcubamba province has an average annual temperature of 27.5°C and an average rainfall of 58 mm/year²⁵ and houses 87.4% of the goat population of the region.²⁶ The producers with the most significant number of goats were selected.

Animals and production system

The study included male and female Creole goats of varying ages and body conditions. Age was estimated based on dentition, ranging from milk teeth (< 1 year) to full mouth (≥ 4 years) and then was ranged into 3 groups, at least 1 year, between 1 and 2 years, and more than 2 years. More than 70% of the sampled goats were over 1 year old. Body condition scores ranged from 2 to 4, with 84% of the goats scoring 3. Among the female goats, 58% were pregnant, while 42% were not; 2% had had an abortion at least once in their reproductive life. All goats were managed under an extensive production system, entirely fed by grazing. Potable water was sourced from the Utcubamba River, Marañón River, and Cruz Baja Spring. None of the goats in the study had been vaccinated against *N caninum* or BTV. Creole animals are important because of their resistance to disease, longevity, and ability to survive with minimal nutritional needs.^{27,28} Moreover, the Creole breed is considered a genetic reservoir of a species on which humans have exerted intense selection pressure.²⁹

Producer characterization

A survey with 54 questions was administered to goat producers in Utcubamba province to characterize their production system and relate it to the prevalence of *N caninum* and BTV. The survey was designed to gather information on (1) the socioeconomic characteristics of the producers, including gender, age, occupation, education level, length of time raising goats, flock size, labor, support received, and any training or technical assistance on livestock health; (2) technical aspects of production, such as husbandry practices, health and reproductive management, nutritional strategies, pasture management, and infrastructure; and (3) environmental conditions. The questionnaire was administered face-to-face directly with the producers, allowing more accurate and detailed information to be obtained. This also helped to clarify any doubts that might arise during the survey and to ensure that the questions were correctly understood. The questionnaire was distributed directly in the field to the selected producers where blood sampling took place during the same day or days after sampling. A group of experts performed validation.

Sample collection and serological analysis

Approximately 4 mL of blood was collected using sterile red cap Vacutainer tubes via jugular venipuncture from 48 males and 306 female goats.

Sampling was performed in March 2023, during the dry season, with consent from the producers. Tubes were labeled with the identification codes of the animals and transported in a polyurethane cooler with ice packs, maintaining an average temperature of 5°C to facilitate coagulation and serum separation. Samples were delivered within 24 hours to the Infectious and Parasitic Diseases Laboratory at the National University Toribio Rodríguez de Mendoza of Amazonas. The serum was separated by centrifugation (Hettich EBA 200) at 3,000 rpm for 3 minutes. The separated serum was stored in 0.2-mL cryotubes, appropriately labeled, and kept at -20°C until further analysis.

Serological testing for antibodies against *N caninum* was conducted using a competitive ELISA kit for neosporosis (iD.vet). For BTV detection, a competitive ELISA kit for anti-VP7 antibodies in serum or plasma from multiple species was utilized (iD.vet). Testing was conducted following the manufacturer's instructions. Readings were performed using an ELISA microplate reader (Bio-Ras iMark) at an optical density (OD) of 450 nm. Positive control and negative control plates were duplicated in each microplate reading, and their values were used to validate the assay. The OD values obtained were analyzed using ID Soft software.

Interpretation of *N caninum* and BTV results was based on the following formula:

$$\frac{S}{N}\% = \frac{OD \text{ sample}}{OD \text{ NC}} \times 100$$

where S is sample and N is negative control.

For *N caninum*, samples with an $\frac{S}{N}\% < 50\%$ were considered positive, those with an $\frac{S}{N}\% > 50\%$ but $\leq 60\%$ were considered doubtful, and samples with an $\frac{S}{N}\% > 60\%$ were considered negative. For BTV, samples with an $\frac{S}{N}\% < 40\%$ were considered positive, while those with an $\frac{S}{N}\% \geq 40\%$ were considered negative.

Statistical analysis

Survey data were compiled into an Excel spreadsheet for analysis. Cluster analysis was performed using R version 4.1.3 (The R Foundation). First, the dendrogram was constructed to identify the number of clusters, then it was validated by performing the hypothesis test (ANOVA, $P < .05$) to compare whether the groups were distinct. The stability of the model was checked with the Ward method and the Euclidean distance. The `fviz_dist()` function was utilized for this analysis, and clusters were visualized with the `fviz_dend()` function from the *factoextra* package³⁰ to identify producer groups with similar characteristics. Associations between variables were examined using the χ^2 test ($P < .05$) based on the identified groups.

Categorical data are presented as frequencies and percentages. Seroprevalence was calculated as the proportion of seropositive animals relative to the total number of sampled animals. The χ^2 test was used to assess significant differences between variables.

Results

Producer characterization

Cluster analysis identified 3 groups of goat producers with similar characteristics (**Figure 2**). Group I included 6 producers, group II comprised 5 producers, and group III consisted of 7 producers.

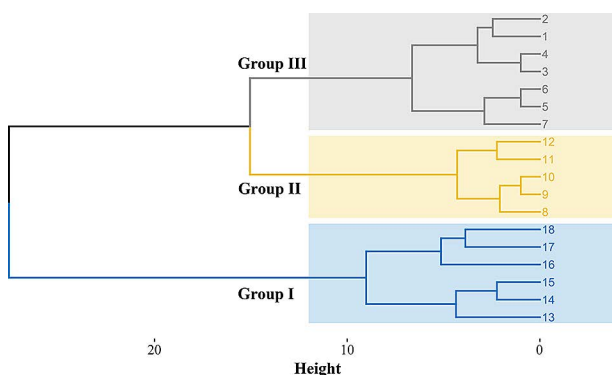


Figure 2—Dendrogram of cluster analysis and Euclidean distance for 18 goat producers Utcubamba province, Amazonas, Peru. Each color belongs to a group of goat producers. Group I belongs to independent producers, and groups II and III belong to associated producers.

All producers utilized an extensive grazing system with natural pastures and no supplemental feeding. Group II has 100% of producers with less than 21 goats, while group III has 85.7% in this category, and group III has 14.3% of producers with more than 60 goats. None of the producers conducted disease diagnostics, and natural mating was the sole reproductive method. Group III is characterized by having corrals and feeding troughs compared to group II, where only 33% of producers have this infrastructure. Infrastructure for goat farming in all groups primarily used wood, and the farming practices were entirely familial. Additionally, none of the producers received financial assistance from the Peruvian government.

There were no significant differences in vaccination practices among the groups ($P > .05$; **Table 1**). However, a higher incidence of the presence of abortion was observed in groups II and III compared to group I, where some producers received technical assistance from government entities, which was reported to aid in goat management. Additionally, group I used river water as a drinking source for their animals, while groups II and III used piped water.

Prevalence of *N caninum* and BTV

The prevalence of *N caninum* and BTV among identified producers' groups is presented (**Table 2**). Significant differences were observed for BTV among the sampled producer groups ($P < .05$). The overall prevalence of *N caninum* was 6.21% (22/354), with detections in 2 of 3 producer groups. The highest prevalence was observed in group II at 7.69%. In contrast, the overall prevalence of BTV was 7.06% (25/354),

Table 1—Goat producer characterization in the tropical dry forest of Utcubamba, Amazonas, Peru.

Variable/levels	Group I (%)	Group II (%)	Group III (%)	P value
Herd size				
< 21 goats	0 (0)	5 (100)	6 (85.7)	< .01
21 to 40 goats	2 (33.3)	0 (0)	0 (0)	
41 to 60 goats	0 (0)	0 (0)	1 (14.3)	
> 60 goats	4 (66.7)	0 (0)	0 (0)	
Goats have been vaccinated				
Yes	1 (16.7)	0 (0)	0 (0)	.35
No	5 (83.3)	5 (100)	7 (100)	
Abortions				
Yes	3 (50)	5 (100)	7 (100)	< .05
No	3 (50)	0 (0)	0 (0)	
Deworming goat management				
Yes	1 (16.7)	0 (0)	0 (0)	.35
No	5 (83.3)	5 (100)	7 (100)	
Deworming dog management				
Yes	1 (16.7)	0 (0)	0 (0)	.35
No	5 (83.3)	5 (100)	7 (100)	
Male breeding origin				
External	1 (16.7)	0 (0)	0 (0)	.35
In house	5 (83.3)	5 (100)	7 (100)	
Infrastructure				
Pens	3 (50)	0 (0)	0 (0)	<.05
Pens and troughs	3 (50)	5 (33.3)	7 (46.7)	
Water supply				
River	6 (100)	0 (0)	0 (0)	<.01
Piped water	0 (0)	5 (100)	7 (100)	
Drinking infrastructure				
River	3 (50)	0 (0)	0 (0)	<.01
Mobile trough	3 (50)	0 (0)	0 (0)	
Fixed trough	0 (0)	5 (100)	7 (100)	
Recent technical assistance				
Yes	3 (50)	0 (0)	0 (0)	<.05
No	3 (50)	5 (100)	7 (100)	
Technical assistance source				
Government base	3 (100)	0 (0)	0 (0)	
Improvement due to technical assistance				
Yes	3 (100)	0 (0)	0 (0)	

found in all producer groups. The highest prevalence for BTV was also in group II, reaching 23.08%.

The prevalence of *N caninum* by sex and age, where the highest prevalence was 6.80% (7/103) in goats aged 1 to 2 years within group I (P value < .05), is presented (**Table 3**). Although no statistically significant differences were observed for sex, the highest numerical prevalence was found in females from group I at 5.88% (18/306).

The prevalence for BTV by sex and age, with findings of significant statistical differences for both sexes and for ages between 1 and 2 years and over 2 years within the groups of goat producers, is presented

Table 2—Prevalence of *N caninum* and bluetongue virus in goats by producer group in the tropical dry forest of Utcubamba, Amazonas, Peru.

Disease/group	Sample size	Positive (%)	95% CI	P value
<i>N caninum</i>				
Group I	254	19 (7.48)	4.68–11.62	.42
Group II	39	3 (7.69)	2.00–21.97	
Group III	61	0 (0.00)		
Total	354	22 (6.21)		
Bluetongue virus				
Group I	254	6 (2.36)	0.96–5.31	<.01
Group II	39	9 (23.08)	11.71–39.72	
Group III	61	10 (16.39)	8.55–28.55	
Total	354	25 (7.06)		

Numbers in parentheses represent the percentage of positive cases.

Table 3—Prevalence of *N caninum* by sex and age in goats by producer group in the tropical dry forest of Utcubamba, Amazonas, Peru.

Variable/level	Sample size	Group I (%)	Group II (%)	Group III (%)	Total (%)	P value
Sex						
Female	306	18 (5.88)	2 (0.65)	0	20 (6.54)	.07
Male	48	1 (2.08)	1 (2.08)	0	2 (4.17)	.53
Age						
< 1 y	107	4 (3.74)	0	0	4 (3.74)	.43
1 to 2 y	103	7 (6.80)	3 (2.91)	0	10 (9.71)	<.05
> 2 y	144	8 (5.56)	0	0	8 (5.56)	.26

Numbers in parentheses represent the percentage of positive cases.

Table 4—Prevalence of bluetongue virus by sex and age in goats by producer group in the tropical dry forest of Utcubamba, Amazonas, Peru.

Variable/level	Sample size	Group I (%)	Group II (%)	Group III (%)	Total (%)	P value
Sex						
Female	306	6 (1.96)	8 (2.61)	8 (2.61)	22 (7.19)	<.01
Male	48	0	1 (2.08)	2 (4.17)	3 (6.25)	<.05
Age						
< 1 y	107	0	1 (0.93)	2 (1.87)	3 (2.80)	.07
1 to 2 y	103	0	1 (0.97)	1 (0.97)	2 (1.94)	.01
> 2 y	144	6 (4.16)	7 (4.86)	0	13 (9.03)	<.01

Numbers in parentheses represent the percentage of positive cases.

(Table 4). The highest prevalence was noted in males in group III with 4.17% (2/48) and in those over 2 years of age in group II with 4.86% (7/144).

Discussion

This study represents the first comprehensive assessment of goat producers in the tropical dry forest of Amazonas, Peru, focusing on their association with the prevalence of *N caninum* and BTV.

We identified 3 distinct groups of producers, with group I and group II showing notable *N caninum* prevalence rates of 7.48% and 7.69%, respectively. *Neospora caninum* was detected in goats from 4 of the 6 herds sampled in group I and 3 of the 5 herds sampled in group II. Previous studies in goats from Piura, northern Peru, reported an average prevalence of 3.32%.¹⁴ Higher seroprevalence rates of *N caninum* have been observed in countries such as Argentina (40.3%),³¹ Ecuador (12.11%),³² and Brazil (26.11%),³³

while China (8.55%)³⁴ and Spain (7.05%)³⁵ reported prevalence levels like those in our study. In contrast, lower seroprevalence rates were reported in Iran (6.22%),³⁶ Italy (5.80%),³⁷ and Romania (2.34%).³⁸

Although the main route of transmission is through vectors such as *Culicoides*,¹⁵ it has been documented that the virus can be transmitted vertically in some cases. Goats infected during gestation can transmit the virus to their offspring. This can occur through the placenta³⁹ or through breast milk,⁴⁰ although the efficiency of this transmission can vary.

Transmission linked to environmental exposure considers factors such as altitude, meteorological conditions such as temperature and humidity, and vegetation. At higher altitudes, propagation is slower; regions with warm temperatures and high humidity are more prone to BTV outbreaks, and likewise, host availability is a key factor in the spread of the disease.⁴¹ On the other hand, sewage ponds, with gentle slopes, free of vegetation and enriched

with animal manure, are mainly permanent water sources that provide excellent year-round development sites for the vector and promote a high level of vector-host interaction.^{42,43} The presence of *N caninum* in group II and its absence in group III, although both share grazing areas and water sources, supports the notion that vertical transmission is the primary mode of propagation in these groups. This finding suggests that observed abortion problems may be attributed to other factors or diseases rather than solely to *N caninum*. Another consideration is that group II goats may be more prone to ingest oocysts due to their grazing behavior; feeding in specific areas where there is a higher concentration of oocysts, pasture conditions; denser or wetter pasture may retain more oocysts; increasing the exposure of the goats and also due to a weaker immune response to this disease.⁴⁴

The classification of goat producers into 3 groups in this study revealed associations with abortion rates and the lack of training and technical assistance regarding animal health. It was observed that all producers in groups II and III reported abortions in their herds. In contrast, producers in group I, who received training and technical assistance, did not report issues. However, this variable was not related with the higher or lower prevalence of *N caninum* and BTV in goats from Amazonas.

In this study, females exposed the highest overall prevalence of *N caninum*. However, within groups, males in group II reported the highest prevalence. In contrast, a study³⁶ in Iran revealed significant disparities between sexes, with males showing a higher prevalence of 16.7%. This suggests that, in our study, females are more susceptible to the disease than males.

The prevalence according to age ranges, it was found that goats older than 1 year had a higher probability of being infected with *N caninum*, consistent with findings from a study³⁵ conducted in Spain. This increased susceptibility in older animals may be attributed to longer exposure to the environment.⁴⁵ Conversely, a study³⁶ conducted in Iran found that goats younger than 1 year were more susceptible to *N caninum*.

The overall prevalence of BTV in the Amazonas region was 7.06%, lower than a previous report²⁴ from the same geographical area (31.3%; 95% CI, 8.3 to 69.5). This earlier study had a smaller sample size, which may affect the reliability of comparisons. Another study²³ in northern Peru's dry forests and arid ecosystems reported a higher prevalence of 23.8% (95% CI, 19.84 to 28.16), with regional variations ranging from 6.0% to 34.9%.

Studies in South America have documented variable prevalences of BTV in goats, such as 5.9% in Minas Gerais, Brazil⁴⁶; 1.8% in Paraíba, Brazil⁴⁷; 4% in Guyana; and, according to Mantilla, 7.4% in Ecuador.⁴⁸ The prevalence rates documented in these studies are notably lower than those observed in the present study. Specifically, groups II and III showed prevalence rates of 23.08% and 16.39%, respectively, higher values than group I (2.36%). These differences could

be attributed to environmental conditions, as groups II and III are situated in ecosystems with higher humidity than group I. This supports previous assertions regarding the impact of temperature and humid environments on the presence of disease vectors.²⁴

Our study also found a higher prevalence of BTV in females (7.19%) compared to reports from China, where males had a higher prevalence (26.7%).⁴⁹ The association between BTV prevalence and age was significant, with older goats showing greater seropositivity, as reported by several authors.^{24,50-52}

This study detected seropositive goats for *N caninum* and BTV in the tropical dry forests of Utcubamba, Amazonas, Peru. We identified 3 distinct groups of producers according to a survey that categorized them based on social, technical (production, health, reproduction, nutrition, infrastructure), environmental, and economic factors. The highest prevalence of *N caninum* and BTV was found in producers from group II, with 7.69% and 23.08% respectively. Despite inadequate animal health management, the overall low prevalence of these diseases suggests that they might not be endemic to this region. As an exploratory study providing evidence of the presence of *N caninum* and BTV in goats from the tropical dry forest of the northern of Peru, it is recommended to eliminate sick animals due to their reduced number, increase vector control and carry out deworming of dogs, all of this without requiring the use of vaccines. In addition, a more constant epidemiological control is suggested to implement policies for controlling and eradicating infectious diseases.

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Disclosures

The authors have nothing to disclose.

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