

Evaluation of the presence of *Sarcocystis* spp. in dogs raised in alpaca farm enterprises in Cajamarca, Peru

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Abstract

The apicomplexans of the genus *Sarcocystis* are parasites that infect various animal species, including humans. Both dogs and other carnivores can act as definitive hosts of this protozoan. The present study aimed to determine the frequency of *Sarcocystis* spp. in domestic dogs raised in three alpaca breeding areas in Cajamarca. Random fecal samples were collected from 102 dogs of different ages and both sexes in three locations related to alpaca breeding: Atahualpa Jerusalén Agricultural Workers Cooperative - Cajamarca (N = 35), SAIS Huacraruco - San Juan (N = 33), and Alpacas Project - Foncreagro - Sorochuco (N = 34). The samples were processed using the Sheather flotation technique. Through microscopic observation, ellipsoidal, light gray sporocysts with average dimensions of 13.87 ± 1.61 μm in length and 9.12 ± 1.05 μm in width were identified. In the data analysis, an overall frequency of *Sarcocystis* spp. of 42.16% (95% CI: 32.57 - 51.74) was determined, with a higher presence in the dogs from Huacraruco ($P < 0.05$). The age and sex of the dogs did not statistically influence the presence of *Sarcocystis* spp ($P > 0.05$). In conclusion, this study reveals a high prevalence of *Sarcocystis* spp. in domestic dogs from the studied areas in Cajamarca. This emphasizes the pressing need to explore practical and optimal control measures.

Keywords: apicomplexan, *Canis lupus familiaris*, protozoan parasite, *Sarcocystidae*, sarcocystosis

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Introduction

In regions where South American camelids are bred, this disease takes on special significance due to the economic importance of these animals in the lives of local communities (Regensburger *et al.*, 2015; Moré *et al.*, 2016). South American camelids, such as alpacas, are raised by families in high-altitude areas and play a fundamental role in family economies, providing high-quality meat, leather, and fiber (McGregor, 2006; Romero *et al.*, 2017). However, sarcocystosis has proven to be a significant obstacle for this industry, as sarcocysts affect the muscles of these animals, deteriorating the quality of their meat and causing considerable economic losses (Carletti *et al.*, 2013; Rooney *et al.*, 2014).

Alpaca breeding in Peru has been hampered by *Sarcocystis* spp., with infections nearing 100% in this species (Castro *et al.*, 2004; Rodríguez *et al.*, 2023). It has been observed that herding dogs in alpaca breeding areas play a significant role in the transmission of these parasites to alpacas (Choque *et al.*, 2007). These dogs, commonly used to guard alpaca and llama herds, are often infected with *Sarcocystis* spp., which could favor the persistence of the parasitic cycle and impact the health of the animals.

Sarcocystosis, a disease caused by intracellular protozoan parasites of the genus *Sarcocystis* (Apicomplexa: *Sarcocystidae*), represents a global concern due to its widespread distribution and economic impact on the livestock industry (Dubey *et al.*, 2015). This group of parasites has a complex life cycle involving definitive hosts, such as carnivores and omnivores, that become infected through the consumption of muscular/cardiac tissues containing the parasites. These hosts release oocysts/sporocysts into the environment, which infect intermediate hosts, including herbivores, where the parasites develop in various tissues, including skeletal muscles, tongue, neck, diaphragm, legs, cardiac muscles, smooth intestinal muscle, and the central nervous system (Fitzgerald *et al.*, 1993; Dubey *et al.*, 2015; Sazmand and Joachim, 2017). Human infections can cause symptoms ranging from enteritis to extraintestinal manifestations with a wide clinical spectrum (Rosenthal, 2021).

Sarcocysts found in South American camelids have been classified under various names, such as *S. aucheniae*, *S. lamacanis*, and *S. lamacenis* (Taylor *et al.*, 2007). Currently, according to standard nomenclature, *S. aucheniae* is proposed as the only valid name for the species of *Sarcocystis* that forms macroscopic sarcocysts in llamas and alpacas (Dubey *et al.*, 2015). Microscopic cysts have also been described in alpacas, llamas, and guanacos from South American countries, and the parasite has been named *S. masoni* (Moré *et al.*, 2016). Cardiac sarcocystosis is an endemic disease in Peruvian alpacas (Rosenthal, 2021). Consumption of inadequately cooked alpaca heart muscle infected with *Sarcocystis masoni* n. sp. (previously known as *S. lamacanis*) can lead to a foodborne illness syndrome in humans (Rodríguez *et al.*, 2023).

The present study was conducted to identify and determine the frequency of sporocysts of *Sarcocystis* spp. in domestic dogs (*Canis lupus familiaris*) raised in three alpaca-influenced areas in Cajamarca:

Cooperativa Agraria de Trabajadores Atahualpa Jerusalén, SAIS (Sociedad Agrícola de Interés Social) José Carlos Mariátegui, and Proyecto Alpacas Foncreagro.

Materials and Methods

The present study was conducted in locations dedicated to alpaca breeding in the Cajamarca region, Peru. The first area corresponds to the Cooperativa Agraria de Trabajadores Atahualpa Jerusalén in Porcón, at an altitude of 3120 meters above sea level (masl) and with temperatures ranging from 4 to 18 °C. The second study area is located in SAIS José Carlos Mariátegui in Huacraruco, covering altitudes ranging from 2800 to 4216 masl, with recorded temperatures between 2.9 and 12.6 °C, and situated in the district of San Juan, Cajamarca province. The third evaluated zone corresponds to the Proyecto Alpacas Foncreagro in Sorochuco, located at 3608 masl, with a temperature range of 7.7 to 21 °C, in the district of Sorochuco, Celendín province (Figure 1).

Due to the limited population of dogs under observation, sampling included all the dogs in each location. These dogs were part of the communities engaged in alpaca breeding and provided companionship to local families in the areas of alpaca breeding and their surroundings.

Following informed consent from dog owners and considering necessary biosecurity measures, external stimulation of the dogs' anal sphincter was performed using gentle finger massages with glycerin-lubricated index fingers. The objective was to obtain fecal samples directly from the rectum. These fecal samples were collected in appropriately labeled polyethylene bags and placed in a styrofoam box for transportation.

Dogs in the three areas lived freely and could roam at any time and in any place. Their defecation habits were primarily observed on the sides of roads and in pastures.

The analyses were conducted in the Laboratorio de Inmunología e Investigación of the Facultad de Ciencias Veterinarias at the National University of Cajamarca within 24 hours after sample collection.

The samples were processed using the sugar flotation concentration technique (Sheather, 1923). Subsequently, observations were made using an optical microscope with a 10X eyepiece and a 40X objective lens. *Sarcocystis* sporocysts were identified based on their morphometric characteristics, following reference guidelines (Miró, 1999; Saeed *et al.*, 2018). Sporocysts were measured using a micrometric eyepiece at 40X, and the values obtained were multiplied by the correction factor of 2.45 corresponding to this specific magnification.

The data were organized in Microsoft Excel, and the frequency and its 95% confidence interval were calculated. Subsequently, statistical analysis was performed using SPSS version 27.0.1 (USA) software to determine the influence of location, age, and sex on the presence of *Sarcocystis* spp. This analysis was conducted using the Kruskal-Wallis test. In cases where statistical differences were found, the Mann-Whitney U test was employed to identify the group

that exhibited differences. A significance level of $P<0.05$ was considered.

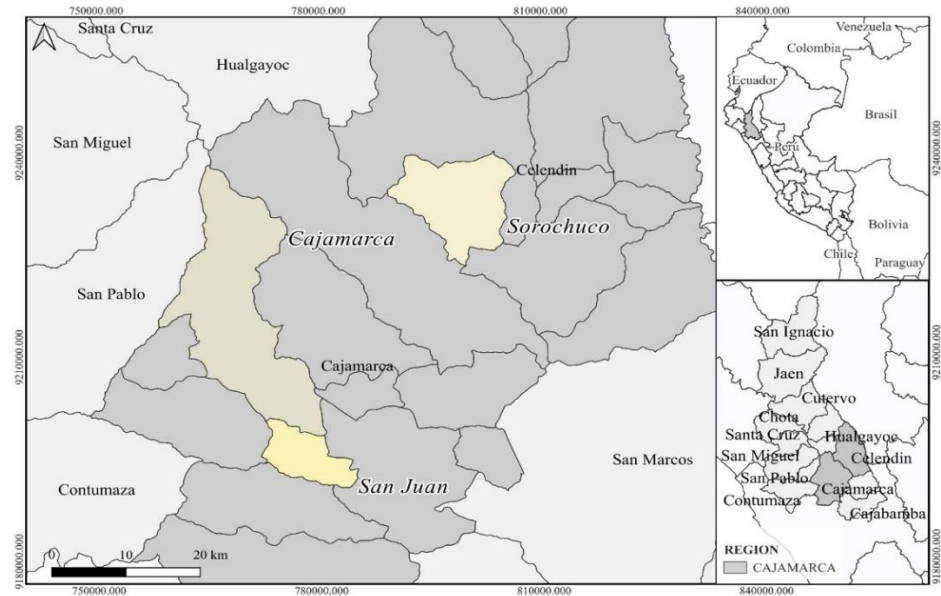


Figure 1 Location of the three assessed alpaca breeding zones in Camajarca, Peru.

Table 1 Frequency (%) of *Sarcocystis* spp. oocysts in the feces of dogs raised in three alpaca-influenced zones in Cajamarca, Peru.

Variable	Category	N	Positives	Frecuency (CI 95%)
Place	Porcón	35	11	31.43 (16.05 - 46.81) ^a
	Huacararuco	33	22	66.67 (50.58 - 82.75) ^b
	Sorochocho	34	10	29.41 (14.10 - 44.73) ^a
Age	< 1 year	44	19	43.18 (28.55 - 57.82) ^a
	> 1 year	58	24	41.38 (28.70 - 54.05) ^a
Sex	Female	43	16	37.21 (22.76 - 51.66) ^a
	Male	59	27	45.76 (33.05 - 58.48) ^a
TOTAL		102	43	42.16 (32.57 - 51.74)

^{a,b} Different letters indicate statistical differences within each variable (Kruskal-Wallis + Mann-Whitney U Post hoc, $P<0.05$).

Table 2 Morphometry of *Sarcocystis* spp. sporocysts found in the feces of dogs raised in three alpaca-influenced zones in Cajamarca, Peru.

Sporocyst (n = 43)	Length (µm)	Width (µm)	Characteristics
Mean	13.87	9.12	Ellipsoidal, light gray (Figure 2)
Mode	14.7	9.8	
Standard deviation	1.64	1.05	
Variance	2.70	1.09	
Minimum	11.03	7.35	
Maximum	17.15	11.03	

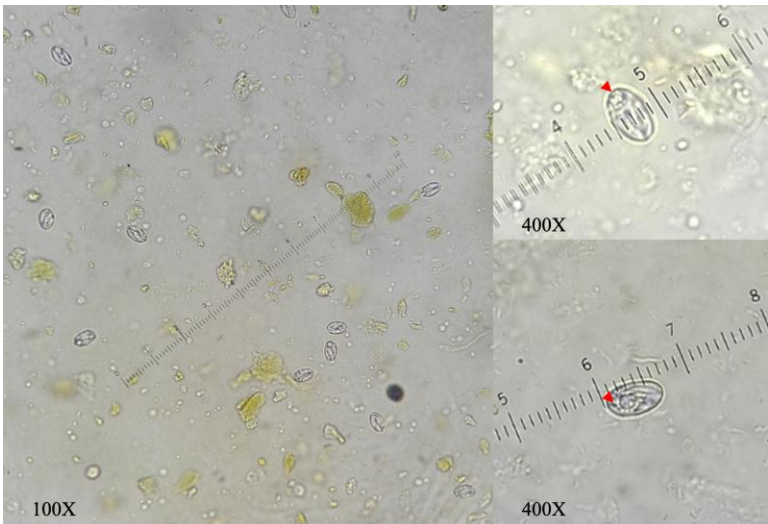


Figure 2 Sporocysts of *Sarcocystis* spp. They appear ellipsoidal, sporulated (sporozoites), and with dispersed granular residue at the poles inside (arrowhead).

Result

A total of 102 fecal samples from dogs were collected from the three locations: N = 35 from the Cooperativa Agraria de Trabajadores Atahualpa Jerusalén – Cajamarca, N = 33 from SAIS José Carlos Mariátegui in San Juan, and N = 34 from the Proyecto Alpacas Foncreagro in Sorochuco, Celendín.

Upon processing and analyzing the data, a general frequency of *Sarcocystis* spp. was determined to be 42.16% (95% CI: 32.57 – 51.74), with a significantly higher presence in the feces of dogs from Huacraruco ($P < 0.05$). There was no statistically significant influence of age and sex of the dogs on the presence of *Sarcocystis* spp. ($P > 0.05$) (Table 1).

Microscopic examination of the samples revealed the presence of ellipsoidal sporocysts of light gray color. The measurement of $n = 43$ sporocysts gave an average of $9.12 \pm 1.05 \mu\text{m}$ width by $13.87 \pm 1.61 \mu\text{m}$ length (Table 2).

Discussion

Through microscopic observation of the samples, ellipsoidal sporocysts with characteristics of *Sarcocystis* spp. were identified. However, molecular studies are required to determine the specific species of *Sarcocystis*. This parasite was observed in 43 of the 102 analyzed dog fecal samples (42.16%). Additionally, no significant differences were observed in the presence of the parasite based on the sex or age of the dogs ($P > 0.05$).

Morphologically, it is not possible to definitively identify the species of a parasite. In the case of *Sarcocystis*, within a specific host, numerous species overlap in terms of dimensions. The values found ($13.87 \pm 1.64 \times 9.12 \pm 1.05 \mu\text{m}$) are similar to those reported by other authors. Most canine sporocyst species have dimensions around $15 \times 10 \mu\text{m}$ (Dubey et al., 2015). Dogs fed on infected camelid meat have been found to harbor sporocysts ranging from $14.6 - 15.0 \times 10.4 - 10.6 \mu\text{m}$ (Saeed et al., 2018). Measures of $13 \times 8 \mu\text{m}$ have also been recorded (Miró, 1999). Furthermore, like in the present study, sporulated oocysts are colorless, have thin walls ($< 1 \mu\text{m}$), and contain 2 elongated sporocysts. Each sporocyst houses 4 slender sporozoites and a granular sporocyst residue, which can be compact or dispersed (Dubey et al., 2015).

However, only dogs fed infected camelid meat have been shown to produce sporocysts in their feces; thus, the dog is considered the definitive host of *Sarcocystis aucheniae* (Schnieder et al., 1984; Cornejo et al., 2007; Zacarías et al., 2013). On the other hand, the definitive host of *Sarcocystis masoni* is still unknown, although it has been suggested that dogs and other canids are possible definitive hosts for this parasite (Moré et al., 2016). Nevertheless, further studies on canids and wild animals in the high-altitude alpaca breeding areas and other South American camelids are needed to identify potential additional definitive hosts of sarcocysts affecting these animals.

In rural areas (like this study), dogs play a fundamental role in labor, and their owners often seek to minimize maintenance costs, which translates into limited investment in their well-being and animal health. In contrast, dogs in urban areas tend to receive

greater attention and care. Therefore, in other studies, rural area dogs have been found to have a higher prevalence of infections caused by a wide variety of helminth and protozoan parasites (González-Ramírez et al., 2021; Santos et al., 2021).

There have been a few similar studies to the present one. Maranganí (Cusco) values consistent with those in this research have been reported. In this study, 211 fecal samples from shepherd dogs belonging to alpaca producers in the area were analyzed, revealing a frequency of $42.3 \pm 9.2\%$ (47/111) in the dry season and $72.0 \pm 8.8\%$ (72/100) in the rainy season, with statistical significance of $P < 0.05$ (Choque et al., 2007). Additionally, significant differences were observed based on age group, with a higher prevalence in dogs older than three years. Despite finding a higher frequency of *Sarcocystis* spp. in Sorochuco, further studies are needed to identify factors influencing the prevalence of these coccidia in each location.

Breeding conditions and the level of care may influence the prevalence and latency of *Sarcocystis* spp. in dogs from rural areas, which could lead to the infection of other animals. In general, rural dog owners tend to be unaware of the risk of parasitic infection, and a large percentage of them do not administer antiparasitic treatments (Michalczyk et al., 2019). However, there is no sanitary care or routine antiparasitic treatment that can affect the cycle of *Sarcocystis* spp.; it is only feeding control. Furthermore, shepherd dogs in alpaca breeding areas have been observed to play a significant role in the transmission of *Sarcocystis* spp. to the alpacas themselves (Choque et al., 2007).

Although the present study did not find an association between *Sarcocystis* spp. and age and sex categories, it is important to note that only two categories and a small population of dogs were considered. In other studies, a relationship has been observed between *Sarcocystis* spp. and longevity in both intermediate and definitive hosts. In these cases, *Sarcocystis* spp. has been more frequently detected in older animals, as they have had more time to be exposed to *Sarcocystis* spp. oocysts/sporocysts, regardless of sex (Choque et al., 2007; Omer et al., 2017).

It is important to note that dogs raised in rural areas tend to defecate anywhere, primarily away from homes, such as in the fields where pastures for herbivores grow. Thus, the alpaca, as an intermediate host, becomes infected with *Sarcocystis* spp. by ingesting sporulated oocysts or sporocysts present in the environment when consuming grass or water. On the other hand, the dog, as a definitive host, acquires the infection by consuming infected meat containing sarcocysts. Once inside the dog, the process of sexual reproduction takes place until oocysts are formed, which are then excreted into the environment through feces, thus contaminating the pastures and water. This completes the parasite's life cycle (Abdel-Ghaffar et al., 2009; Dubey et al., 2015).

It should be noted that the life cycle of *Sarcocystis* spp. is completed due to ecological and behavioral factors, such as the availability and proximity of definitive hosts (carnivores) and intermediate hosts (herbivores and omnivores), as well as environmental conditions (humidity, temperature) that favor the

survival of sporocysts. Additionally, the feeding behavior of the hosts, particularly the ingestion of raw meat or grass contaminated with feces, plays a crucial role. The migration of animals and the interaction between domestic and wild animals in production systems also contribute to the spread of the parasite (Taylor *et al.*, 2007; Dubey *et al.*, 2015).

The present study has revealed a high prevalence of *Sarcocystis* spp. in domestic dogs within the surveyed areas of Cajamarca. This emphasizes the pressing need to explore practical and optimal control measures. Additionally, it is important to note that only morphometric analysis was conducted to identify *Sarcocystis* spp., and these techniques solely are often insufficient for definitive species identification. Therefore, the lack of molecular analysis to confirm the species was a limitation, as such results would have provided more precise data.

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