Veterinary and Animal Science 18 (2022) 100270

Contents lists available at ScienceDirect

Veterinary and Animal Science

journal homepage: www.elsevier.com/locate/vas

Determination of *Sarcocystis lamacanis* microcysts in the cardiac muscle of alpacas (*Vicugna pacos*) and their correlation with troponin cTnI. A study performed in the high Andean region of southern Peru

Fernando Fernandez-F [a,b], Roxana Gutiérrez-A [b], Víctor Pacheco-S [b], José Chirinos-T [a], Daniel Marcelo Lombardo [c], Luis V.M. Olivera [d], Julio Cesar Bernabe-Ortiz [a], Patricia López-Casaperalta [*,a]

[a] Universidad Católica de Santa María,Urb. San José s/n, Umacollo, Arequipa, 04013, Peru
[b] Universidad Católica de Santa María, Post-Graduate School, Urb. San José s/n, Umacollo, Arequipa, 04013, Peru
[c] Facultad de Ciencias Veterinaria, UB, Av. Chorrarin 280, Buenos Aires, C1427CWO, Argentina
[d] Faculty of Veterinary Sciences, National University of the Altiplano, Av. Floral 1153, Puno,21001, Peru

ARTICLE INFO

Keywords:
Sarcocystis lamacanis
Vicugna pacos
Microcysts
Troponin cTnI
Cardiac muscles

ABSTRACT

The breeding of alpaca (*Vicugna pacos*) is one of the most important economic activities in the high Andean areas of Peru. The commercialization of products derived from alpaca represents more than 80% of the income of high-Andean families. However, the infestation of parasites such as *Sarcocystis lamacanis* in the alpacas causes economic losses that deteriorate the already diminished quality of life of the alpaca breeder. The search for biomarkers that allow the early detection of these parasites is one of the most critical challenges in Peru, a country with the largest population of alpacas worldwide. This work aimed to analyze and quantify the microcysts formed by the parasite and relate them to the troponin cTnI level in the blood serum. Troponins are proteins secreted when there is damage to the cardiac muscle. 60 blood and cardiac tissue samples were collected from Tisco and La Raya slaughterhouses, localities of Caylloma Province in Arequipa, and Chucuito District in Puno, both regions in southern Peru. The cardiac muscle samples were processed with the routine histology technique and stained with hematoxylin and eosin. In addition, serum samples were processed with the ELISA and immunochromatography methods for troponin cTnI. Results were 100% positive for the presence of *Sarcocystis lamacanis* microcysts in all cardiac muscle samples. The average microcyst quantification per field of 100x were 3.5 and 5.7 for the Tisco and La Raya samples. In addition, several microscopic lesions were observed in the cardiac muscles: microcyst infiltration between muscle fibers, basophilic microcysts with a thick outer membrane and bradyzoites inside, and tissue displacement. On the other hand, all serum blood samples were negative for troponin cTnI, with both methods, ELISA and immunochromatography. For results, we infer troponin cTnI do not can be used as a biomarker for heart damage caused by *Sarcocystis lamacanis* parasite in alpacas.

1. Introduction

Nowadays, Peru is the country with the largest population of alpacas worldwide (Contreras Flores, 2019; Gutierrez et al., 2018). It is estimated that the national population of this camelid is close to 4.5 million units (Contreras Flores, 2019; Galiano and Hinostroza, 2022). Due to their geographic conditions, the departments of Puno and Arequipa account for more than half of this population (45.3% and 10.5%, respectively) (Galiano and Hinostroza, 2022). Adaptation to adverse climatic conditions and its high profitability has made alpaca breeding one of the most important economic activities in the high Andean areas of Peru (Avilés-Esquivel et al., 2018; Contreras Flores, 2019; Sillau et al., 1976; Witt and Huerta-Sánchez, 2019). Even more, the alpaca (*Vicugna pacos*) is, together with the llama (*Lama glama*), the two domestic species of South American camelids (SACs) with the highest breeding and commercialization in the country (Gutierrez et al., 2018). However, a large part of the population dedicated to alpaca breeding depends exclusively on this activity, representing up to 80% of their income

* Corresponding author
E-mail address: plopez@ucsm.edu.pe (P. López-Casaperalta).

https://doi.org/10.1016/j.vas.2022.100270
Received 26 August 2022; Received in revised form 16 October 2022; Accepted 2 November 2022
Available online 11 November 2022

(Animal Production &. Health Division, 2008; Ministerio de Agricultura y Riego, 2019). According to the Livestock Plan 2017-2021 of the Ministry of Agriculture and Irrigation (MINAGRI), 73% of alpaca producers are small breeders, of which 45% are considered poor or in extreme poverty (Becerra sánchez et al., 2017).

Unfortunately, this almost total economic dependence on smallholders in alpaca farming can be drastically affected when the animal becomes ill (Arphi Paccaya, 2020; Frenández-Baca, 2005; Scott et al., 2011). One of the diseases that causes the most significant financial loss is sarcocystosis due to its high morbidity in alpacas (Chávez et al., 2008; Saeed et al., 2018). This loss is because the infected animal has sarcocystis in the skeletal and cardiac muscles. Consumption of this meat can lead to mild to severe gastroenteric symptoms in humans, including nausea, diarrhea, cramps, and chills due to a toxin in the cysts (Leguía and Clavo, 1989). This situation leads to the seizure of meat, generating significant economic losses ranging from hundreds of thousands to millions of dollars (Chávez et al., 2008; Frenández-Baca, 2005; Rodríguez et al., 2012).

Sarcocystosis is a disease caused by apicomplexan protozoan parasites of the genus *Sarcocystis*. More than 200 species are currently known (Dubey et al., 2015), of which *Sarcocystis aucheniae* and *Sarcocystis lamacanis* are the recognized species that affect alpacas (Leguía and Clavo, 1989). *Sarcocystis aucheniae* sp. produces macroscopic cysts whose size varies between 0.4 to 8 mm that develop in muscles located in the tongue, esophagus, neck, and intercostals (Saeed et al., 2018). On the other hand, *S. lamacanis* produces microcysts (800 μm in length and 35-95 μm in width) that develop in cardiac muscle tissue and are rapidly maturing (Leguía and Clavo, 1989; Leguía P.and Casas, 1999; More et al., 2016). These parasitic forms infiltrate cardiac muscle fibers, injuring muscle tissue. However, the details of how the damage is done have not yet been well described. Although there are studies that have evaluated the prevalence of *S. lamacanis* in alpacas (Condori-Quispe et al., 2019; Gutiérrez et al., 2015), the description of lesions in the heart, size of microcysts, as well as the quantity or density of presentation is scarce.

Several efforts are being made to prevent the incidence of sarcocystosis in these camelids. An example is the creation of a vaccine that allows the creation of antibodies that interrupt the life cycle of the parasite (Frenández-Baca, 2005; INIA, 2022). However, an exciting proposal is presented by López-Torres et al. (2015), in which they searched for a biomarker that would allow early detection of the disease or its degree of infection by *S. lamacanis* microcysts. For this purpose, they used the enzyme biomarkers cardiac creatine myocardial band (CK-MB), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH). In that same work, troponins are mentioned as excellent biomarkers in diagnosing cardiomyopathies (Maynard et al., 2000). Even more, some studies show the relationship of troponins with heart disease in alpacas (Blass et al., 2011). However, their relationship with sarcocystosis has not been explored.

Troponins are proteins present in skeletal muscle fibers that, in the case of the heart, are responsible for muscle contraction (Chaulin, 2021). Three isoforms of these cardiac troponins are recognized, T (cTnT), I (cTnI), and C (cTnC). T and I isoforms are the only ones of medical interest for diagnosing myocardial lesions (Alastuey et al., 2020; Arboine-Aguirre et al., 2018; Correa and Galvis, 2014; Pereira et al., 2016). As a biomarker, cTnT has been used as a marker for non-invasive medical diagnosis. Its increase correlates with damage to the myocardium in both people and animals (Hemdon et al., 2002). On the other hand, cTnI is a specific cardiac marker primarily used to determine or diagnose acute myocardial infarction (Cala et al., 2015; Medina et al., 2019). Studies have shown that cTnI rises rapidly after cardiomyocyte injury (Mendes et al., 2019). Moreover, cTnI is the one troponin isoform that is found only in cardiac muscle. Experimental tests carried out to measure the number of cTnI in human patients has also been validated in several veterinary species (Connolly et al., 2003). An example is that this marker was observed in cases of lesions of thromboembolism in dogs with myocardial injury caused by other parasites such as leishmaniasis (Canberh and Kerem, 2018). An additional advantage is that cTnI is highly conserved between species (Correa and Galvis, 2014), so it could be a specific biomarker that correlates with damage caused by sarcocystosis-derived microcysts.

The characterization of microcysts caused by the parasite *S. lamacanis* in the cardiac muscle has not been sufficiently explored. In addition, its correlation with the level of troponins would mean finding a specific biomarker in the early diagnosis of this disease. This research aims to address these issues and contribute to the analysis and understanding of sarcocystosis. For this, the quantification of microcysts and the identification of microscopic lesions are presented. In addition, cTnI troponin levels were evaluated by ELISA and immunochromatographic methods in order to correlate them with the presence of microcysts caused by the *S. lamacanis* parasite.

2. Materials and methods

2.1. Location

This study was performed in Tisco and La Raya slaughterhouses, localities of Caylloma, Department of Arequipa, and Chucuito, province of Puno, Department of Puno. Tisco is located northeast of Arequipa at 4188 meters above sea level (masl). It borders the departments of Cusco, to the north, and Puno, to the east. Its population is mainly dedicated to raising and trading alpaca, llama, and trout products. On the other hand, La Raya is located at 4006 masl in the south of Puno. The economic activity of the population is agriculture, livestock, and fishing.

2.2. Animals

Sixty adult alpacas of both genders were selected, of which 31 came from the Tisco and 29 from the La Raya slaughterhouse. The number of alpacas analyzed was limited to the number of animals sacrificed in both slaughterhouses during the month the samples were gotten. For each specimen, control measurements were taken so that the cardiac tissue and blood serum samples matched.

2.3. Sampling and tissue preparation

Samples for histopathology were taken from the cardiac muscle of the alpacas. The approximate size of the tissue samples was 1 cm wide and 2 cm long. Samples were placed in a bottle containing 10% buffered formalin in volume at a concentration of 40 to 1 (formaldehyde and cardiac sample, respectively). Later, histological sections with a thickness of 3 microns were made and stained with Hematoxylin/Eosin. Once the samples were fixed, cut, and stained, the presence of *Sarcocystis lamacanis* microcysts was determined. Microcysts were quantified per optical field at 100x to relate them to the troponins level. This high optical field has been used in various studies, such as searching for human biomarkers or investigating the zoonosis caused by a rodent endemic to South America (Cwirenbaum et al., 2021; Li et al., 2016). These analyses were processed in the Medicine Faculty of the Universidad Nacional de San Agustín (UNSA) of Arequipa.

The blood serum samples were taken from the alpacas before processing in the slaughterhouses. Serum was stored and labeled in yellow vacutainers with a coagulation activator gel, ensuring that the blood samples coincided with identifying the cardiac muscle samples. Once the serum samples were taken, the formation of clot waited and then centrifuged and preserved the serum in refrigeration between $2-7^{\circ}C$ in a thermal box. Finally, all serum samples were transferred to Universidad Católica de Santa María (UCSM) research facilities in Arequipa, where the immunochromatography and ELISA tests would be carried out.

2.4. Determination of troponin I level

The level of the cTnI isoform in blood serum was determined by immunochromatography (qualitative test) and ELISA (quantitative test).

2.4.1. Immunochromatography test

The kit ABON™ cTnI One Step Troponin I Test Device (Whole Blood/ Serum/ Plasma) was used for immunochromatography testing. Specifically, this kit has been used to detect myocardial injuries in sheep (Cunha et al., 2022; Leite, 2020). In this test, the blood serum sample is placed on a membrane containing reagents with which it will form a mixture. By capillary effects, this mixture will move until it comes into contact with the region coated with the anti-cTnI antibody. If there are cTnI levels above 0.11 ng/mL, a colored band will form, indicating that the result was positive for the presence of cTnI. Conversely, the absence of such a band indicates a negative result. As a control procedure, a colored line will always appear in the control band region, meaning that an adequate volume of sample has been added and the membrane reaction has occurred.

Before performing the test, serum samples should be allowed to reach room temperature (15 to 30°C). Once the desired temperature is reached, the immunochromatography kit is opened, and 75 µL should be transferred to the sample well and incubated for 10 minutes at room temperature. Finally, the readings indicated by the coloration of the bands of the device are done.

2.4.2. ELISA test

Samples were analyzed using the Human TNNI3 / Troponin I, Cardiac Muscle ELISA kit (Sigma-Aldrich™), according to manufacturer instructions. The kit contains a split-type reaction plate (12 × 8) coated with mouse monoclonal anti-TnI antibodies; five standard reference sets (0-75 ng/mL) lyophilized TnI; cTnI enzyme conjugate reagent (13 mL/vial) contains monoclonal mouse anti-TnI conjugated to horseradish peroxidase in a Tri-Buffer BSA solution with preservatives; TMB reagent (11 mL/bottle) contains TMB one-step solution, and stop solution (11 mL/bottle) contains dilute hydrochloric acid (HCl 1.0 N). Absorbances were measured with ELISA reader M+H Medical using a wavelength of 450 nm.

2.5. Statistical analysis

The results were analyzed using the statistical system SPSS. Freeman-Halton test, descriptive statistics of frequencies and the Chi-squared test were used to obtain bilateral asymptotic significance.

3. Results and discussion

In the present study, 60 specimens of the species *Vicugna pacos* destined for the commercialization of their meat were analyzed. The specimens were obtained from the high Andean regions of Tisco and La Raya, located in the departments of Arequipa and Puno. The sampling time was 30 days, during which cardiac tissue samples and blood serum were taken from the alpacas sacrificed in the slaughterhouses. In total, 31 samples were obtained from the Tisco and 29 from the La Raya slaughterhouse. All alpacas were adults (>3 years), and gender was not considered in the sampling.

All alpacas underwent a superficial visual inspection in order to detect possible symptoms of the disease. Although sarcocystosis is usually asymptomatic or has no visible signs, studies suggest that there are symptoms that could be associated with the disease. Chávez et al. showed that in alpaca calves infected with *S. lamacanis* sporocysts, symptoms included incoordination, prostration, anorexia, and pyrexia (Chávez et al., 2008). On this inspection, all specimens showed a healthy appearance.

3.1. Prevalence of S. lamacanis in cardiac muscle of alpacas

Histological sections of heart tissues were analyzed using a Leica DM750 microscope and a Leica ICC50W camera. The microcyst count was performed with an optical field of 100x (10 ocular magnification and 10x objective magnification). The results showed the presence of microcysts in all the analyzed tissues (Fig. 1). The heat map depicts a higher density of microcysts per optical field in the samples from the Raya, being the samples in which the highest number of microcysts were observed (21 and 14). On the other hand, in the samples from Tisco, the highest number of microcysts per optical field was 9. The average values obtained were 5.7 (La Raya) and 3.5 (Tisco). Although similar results have been observed in other studies (Guerrero and Hernández, 1967; Mostajo, 1983), the healthy appearance of the animals before slaughter suggested that a lower prevalence would be obtained. In 2015, López-Torres et al. got the same prevalence in their analysis, although their population size was smaller (41 alpacas) (López-Torres et al., 2015). In the same year, with a much larger population (939 alpacas), Gutiérrez et al. obtained a prevalence of 80.62% in a slaughterhouse in Nuñoa, a locality of Puno. Gutiérrez et al. (2015). In 2016, Quispe et al. found a prevalence of *S. lamacanis* of 85.1% in 154 alpacas (Condori-Quispe et al., 2019). These results demonstrate that the high levels of pasture contamination and poor sanitation in the high Andean region of Peru continue.

For the count of microcysts, the results were divided into three groups according to the number of microcysts observed: low number of microcysts, 0-5 (LNM); medium, 6-10 (MNM), and high, >11 (HNM). The total average number of cysts found in the cardiac tissue samples was 4.6±3.8. When separating the sampling by region, in the case of the Tisco slaughterhouse, the average number of counted cysts was 3.5±2.9, and that of La Raya was 5.7±4.3. These results could indicate that the alpacas from the La Raya region were exposed to the *S. lamacanis* parasite sometime before the alpacas from the other group. If ranges analyze the results, it can be seen that 66.7% of the specimens showed a low number of microcysts (LNM, 2.5±1.4 on average). In the case of the MNM group, 28.3% presented an average of 7.6±1.4 microcysts, while the remaining 5.1% (HNM) had an average of 15.3±5.1 microcysts. However, these average values of observed microcysts are low compared to those obtained by López-Torres et al. The researchers found a minimum average of 6.4±3.4 microcysts and a maximum average of 130.3±28.0 per area analyzed (López-Torres et al., 2015). Nevertheless, it is necessary to consider that these values correspond to an area per 4x objective magnification.

Using the Freeman-Halton test, an extension of Fisher's exact test, and the evaluation of 72 tables, a value of $p = 0.198 > 0.05$ was obtained. This result shows that the frequency of the number of microcysts in the cardiac muscles of alpacas from the localities of Tisco and La Raya did not present statistically significant differences. Likewise, Table 1 shows the analyses of microcysts numbers found in the cardiac tissue samples. As seen, in the LNM range, the number of microcysts per optical field in the Tisco samples is higher than in the La Raya samples (77% against 55%). However, for the ranges MNM and HNM, the percentage of incidence in the La Raya samples doubles that of Tisco. This means that there is a greater development of the parasites in the analyzed tissues from La Raya.

3.2. Morphological characteristics of S. lamacanis

To perform the morphological description of the microcysts caused by *S. lamacanis*, all the samples obtained in this study were analyzed. Analyses were carried out using optical microscopy at 100x magnification. Figure 2 shows the histopathological sections of heart tissue for a representative sample obtained at the Tisco slaughterhouse (Fig. 2a) and La Raya (Fig. 2b). In both cases, the microcysts have a basophilic appearance and show lymphocytic infiltration.

Analyses show characteristic microcysts of this parasite (Flores

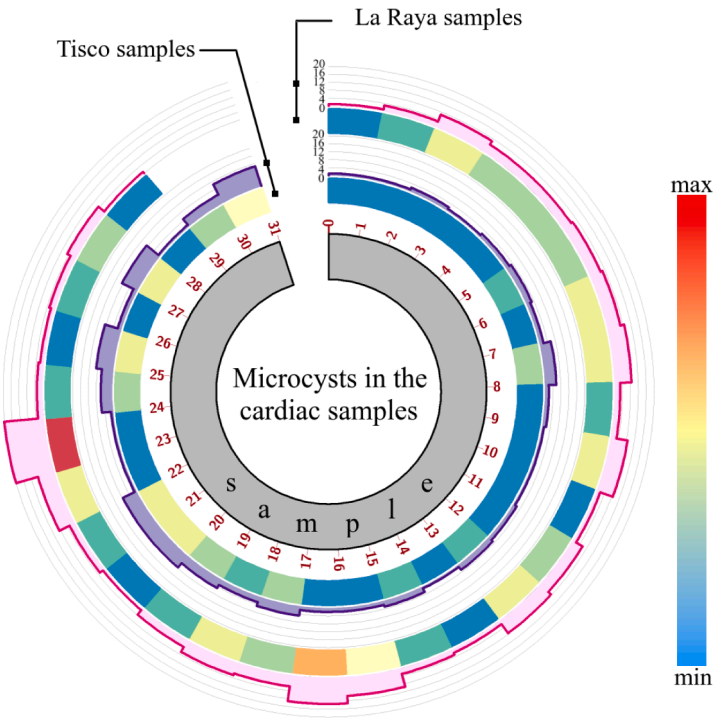

Fig. 1. Heat map and histogram of the count of microcysts found in the cardiac tissues. The scale in the heat map goes from 0 (minimum value, blue color) to 21 (maximum value, red color). In the histograms, the colors only represent where the samples were collected, i.e., purple color for Tisco and magenta for La Raya samples. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Prevalence of *S. lamacanis* according to the microcyst count range in both slaughterhouses.

			Region	
			Tisco	La Raya
Microcysts	1-5	Count	24	16
		% prevalence	77.4%	55.2%
	6-10	Count	6	11
		% prevalence	19.4%	37.9%
	>11	Count	1	2
		% prevalence	3.2%	6.9%
Total		Count	31	29
		% prevalence	100.0%	100.0%

Llanque, 2015; Leguía and Santiago, 2019), fusiform (Fig. 3a), which are characterized by being elongated and narrow in the center; ovals (Fig. 3b), and round (Fig. 3c). These last two forms represent a greater probability of the adjacent muscle fiber deformation. This deformity is related to cardiac tissue damage, which would trigger the myopathy observed in the samples. Another consequence of the presence of this type of microcysts could be the release of troponins in response to the damage suffered by the heart muscle. However, the number of microcysts observed is low, which could mean that the disease is in the early stages of development. In Fig. 3b, the microcyst is found invading Purkinje fibers. So, the stained facilitates observation of the connective tissue (secondary wall) between the cyst and the cardiac fibers.

The displacement of the muscle fibers adjacent to the microcyst is more evident as it increases in size. This displacement could lead to morphological changes in the myocytes, which could lead to Zenker degeneration. These alterations usually present as color changes (the fibers turn pale), fiber enlargement, or globose cells (Cooper and Valentine, 2015; Finlayson et al., 1971; Gutiérrez et al., 2015). Figure 4 shows the displacement of the cardiac muscle fibers due to the presence of fusiform (4 a) and round (4 b) microcysts. In both figures, no degeneration of the myocytes was observed. Moreover, the cysts have a thick capsule and bradyzoites inside, as mentioned by Flores (Flores Llanque, 2015).

The analysis also showed the presence of microcysts in the Purkinje fibers (Fig. 5). A high number of lymphocytes was observed in these areas (Fig. 5a and b). In a close-up of the microcyst (Fig. 5c), the characteristic bradyzoites of this parasite could be observed. The myocyte-microcyst connective walls are also appreciable. In this study, no necrosis lesions were observed in cardiac muscle or Purkinje fibers, which has been observed in previous works (Condori-Quispe et al., 2019; Flores Llanque, 2015).

3.3. cTnI determination in blood serum

Troponin cTnI is widely used as a sensitive and high-specific biomarker in the prognosis and diagnosis of myocardial injury (Chapman et al., 2020; Reichlin et al., 2009). An elevated level of troponins suggests possible damage to the heart muscle that, in its most extreme cases, could lead to the death of the individual. Because its protein structure is highly conserved among mammals, the assays used in its detection have also been used in animals (Fraser et al., 2013; Langhorn and Willesen, 2016; Tharwat, 2012; Tharwat et al., 2013), including the alpaca (Blass et al., 2011). For this reason, two commercial kits for detecting human cTnI have been used in this work. Namely, ABON^{TM} cTnI One Step Troponin I (Whole Blood/Serum/Plasma), and Sigma-Aldrich^{TM} ELISA Kit. Both tests were chosen for their high sensitivity in detecting cTnI levels in blood serum. The immunochromatography test can detect cTnI levels of 1.0 μg/mL, while the ELISA test can detect 0.1 μg/mL.

The tests were conducted considering the whole population of sacrificed alpacas in the two slaughterhouses. The results of both tests are shown in Table 2. Once all the samples were analyzed, it was observed that 100% were positive for the presence of microcysts and 0% for the presence of troponins in the blood serum. Noteworthy, troponin cTnI levels were not detected in the serum samples analyzed, both in the Tisco and La Raya samples. These unexpected outcomes may be because

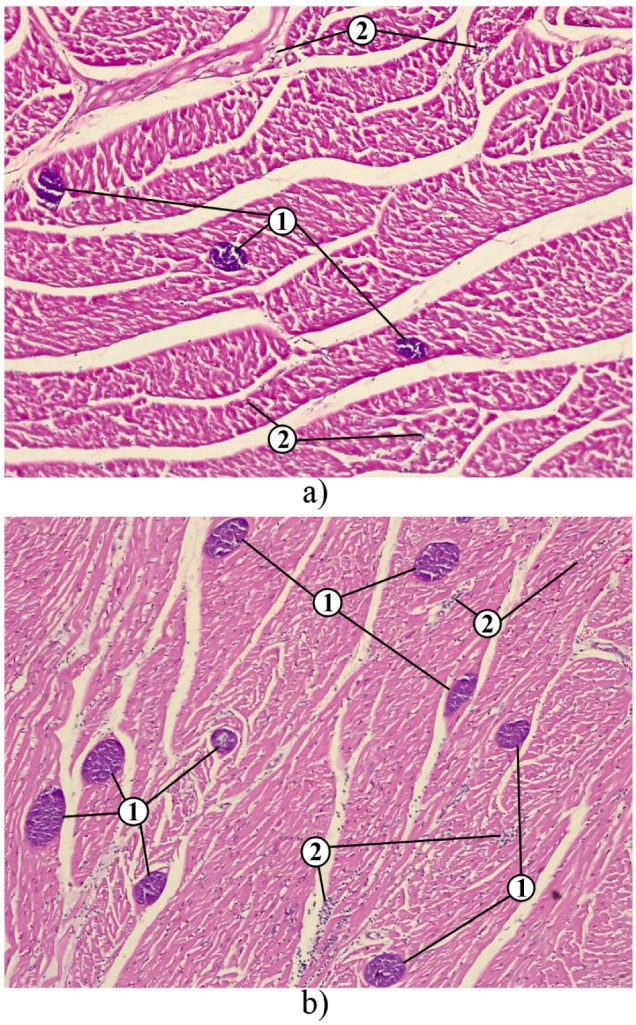

Fig. 2. Microcyst positive sample of *Sarcocystis lamacanis* from (a) Tisco, and (b) La Raya. Numbers indicate 1: microcyst; 2: lymphocytes.

troponins can only be found with lesions or necrosis in cardiac muscle fibers. Troponins are used as specific biomarkers when their levels are above average, and they can predict myocyte injury because of severe ischemia (Álvarez et al., 2012). The negatives in the tests seem to confirm that the sarcocystosis in the analyzed alpacas was still in the early stages since there is no evidence of myocardial ischemia and, therefore, necrosis.

For the chi-square tests, the value obtained from the sampling carried out in Tisco was $\chi^2 = 93.0$, while in La Raya, it was $\chi^2 = 87.0$. Results show the frequency of *Sarcocystis lamacanis* in the different immunological and histological tests present statistically significant differences. Results suggest the troponins cannot be used as predictors of muscle damage because of microcyst infections in alpacas, having a negative correlation, as stated by López-Torres et al. (2015)

3.4. Study limitations

This study has some limitations that may affect our results. Firstly, our sample size was conditioned both by the number of alpacas processed in the slaughterhouses and by the sampling time (30 days). It should be noted that both slaughterhouses belong to regions with low population density. Secondly, based on the results obtained in the microcyst count, we inferred that the sarcocystosis disease was in the early stages of development and the damage to the cardiac muscle was not yet evident. Consequently, the production of troponins in the blood

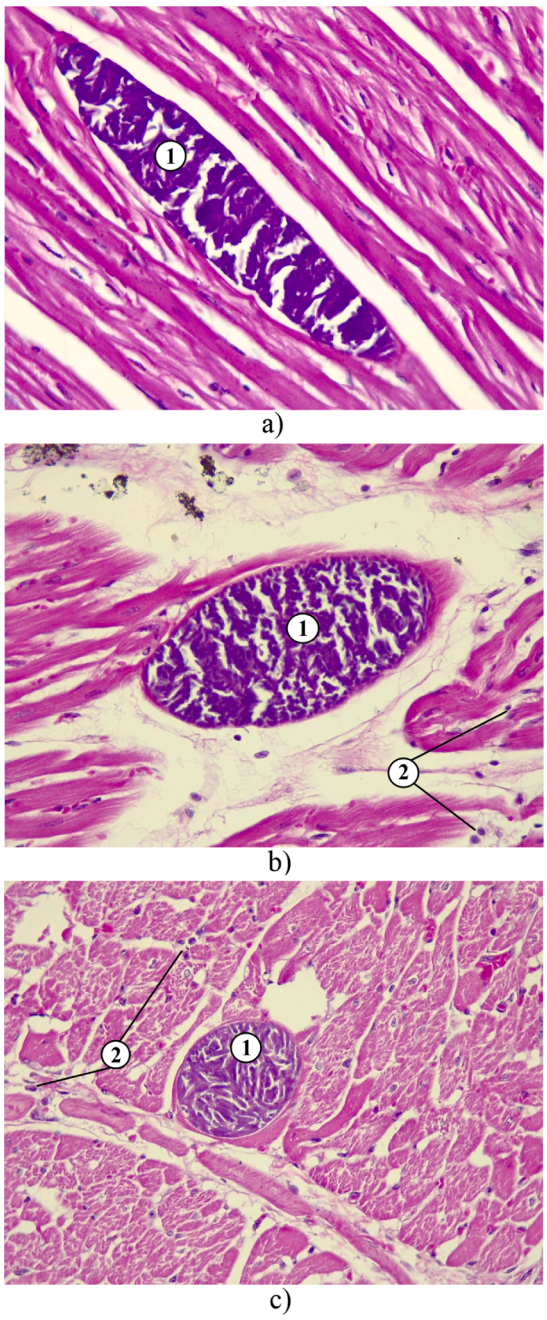

Fig. 3. Morphology of *S. lamacanis* microcysts found in cardiac tissues of the alpacas. a) fusiform; b) oval; and c) rounded forms. Numbers indicate 1: microcyst.

serum would still not be triggered or not at the level necessary to be detected. Finally, no molecular/sequencing assays or genetic analyses were performed in this study to identify the *Sarcocystis spp.* involved in the clinical pictures of sick alpacas. However, *Sarcocystis* spp. is known to be highly specific, and in the case of alpaca, the infection is caused by two species, *S. euchinae* and *S. lamacanis*. Of these species, *S. lamacanis* is the only one that forms microcysts located in the heart muscle. For this reason, we have inferred that *S. lamacanis* sp. was the species observed in our analysis.

4. Conclusions

This work aimed to analyze whether there was a correlation between the presence of microcysts derived from *S. lamacanis* in the heart muscle

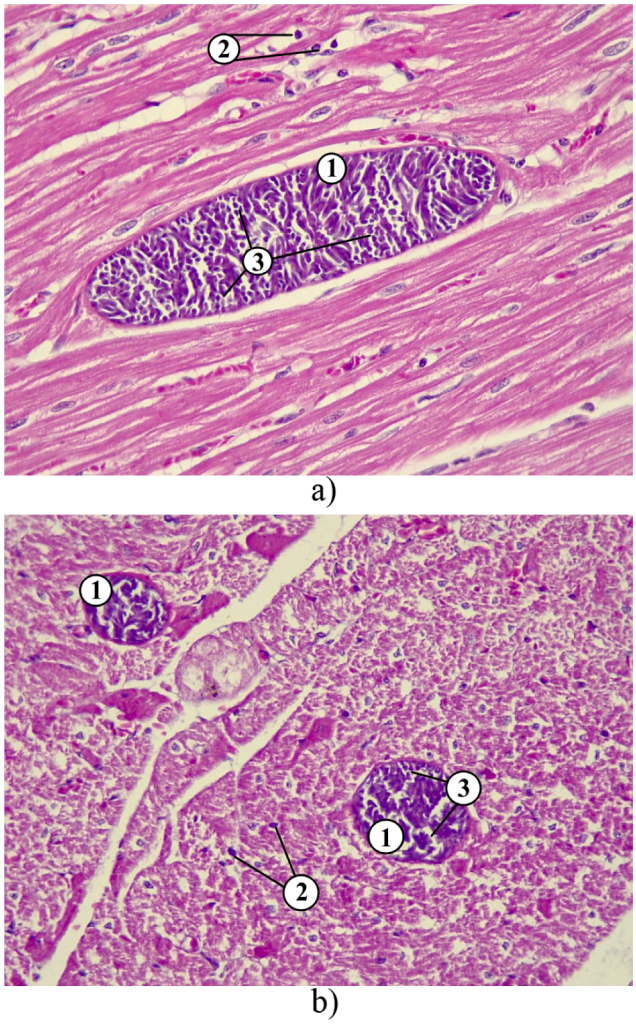

Fig. 4. Image of the bradyzoites inside the microcysts with the following shapes: a) rounded and b) fusiform. The numbers indicate 1: microcyst; 2: lymphocytes; 3: bradyzoites.

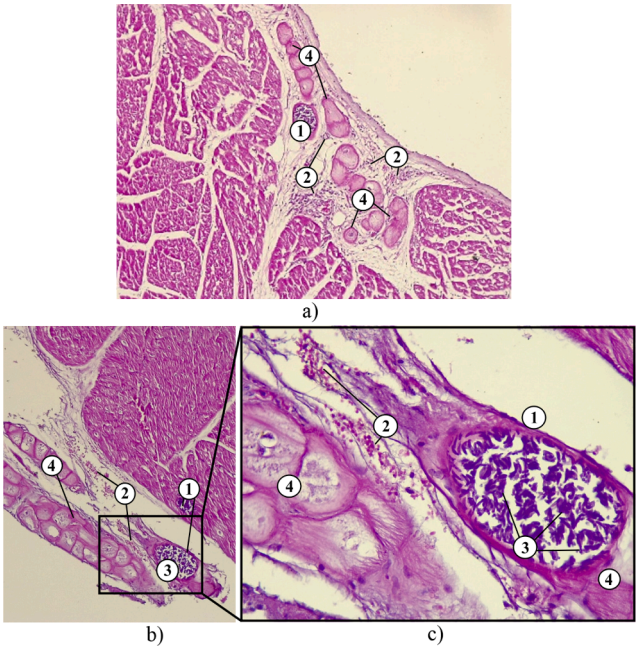

Fig. 5. Microcyst of *Sarcocystis lamacanis* in Purkinje fiber. Numbers indicate 1: microcyst; 2: lymphocytes; 3: bradyzoites; 4: Purkinje fiber.

Table 2
Descriptive statistics of frequencies from Tisco and La Raya samples results.

Dx.	Histopathology		ELISA		ICG	
	No.	%	No.	%	No.	%
Tisco						
Positive	31	100	0	0	0	0
Negative	0	0	31	100	31	100
Total	31	100	31	100	31	100
		χ^2=93.0		$P < 0.05$		
La Raya						
Positive	29	100	0	0	0	0
Negative	0	0	29	100	29	100
Total	29	100	29	100	29	100
		χ^2=87.0		$P < 0.05$		

and the level of the troponin cTnI in the blood of alpacas. We hypothesized that the detection of this troponin could help in the detection of sarcocystosis in these animals without the need to sacrifice them. The results showed to be positive for the presence of microcysts in 100% of the cardiac muscles of the alpacas. However, no significant levels of troponins were detected, so using this biomarker, selective for heart muscle lesions, would not be practical in diagnosing sarcocystosis in alpacas. However, the limitations of our study could have influenced the results obtained. Therefore, a more robust analysis is recommended to determine the usefulness of the troponin cTnI level as a specific biomarker of this disease.

Ethical statement

Funding

This research was financed with the support of the Santa Maria Catholic University of Arequipa, with a grant from the Research Fund of the University (Resolution 25178-2018) organized by the Vice-Rector's Research Office.

Declaration of Competing Interest

The authors declare no conflict of interest.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Fernando Fernandez-F: Conceptualization, Validation, Data curation, Writing – original draft, Writing – review & editing, Methodology, Software. **Roxana Gutiérrez-A:** Validation, Formal analysis, Visualization. **Víctor Pacheco-S:** Software, Formal analysis, Writing – original draft, Data curation. **José Chirinos-T:** Formal analysis, Investigation. **Daniel Marcelo Lombardo:** Methodology, Validation, Resources, Supervision. **Luis V.M. Olivera:** Methodology, Investigation, Visualization. **Julio Cesar Bernabe-Ortiz:** Methodology, Formal analysis, Visualization. **Patricia López-Casaperalta:** Conceptualization, Methodology, Supervision, Project administration, Funding acquisition.

References

Alastuey, M. L., Rodríguez, B. G., Matilla, M. R. G., Perales, C. R. L., & Fernández, E. R. (2020). Elevación persistente de troponina I. *Revista Colombiana de Cardiología, 27* (3), 186–188.

Álvarez, I., Uribe, A., & Duarte, S. (2012). Biomarcadores de la falla cardiaca en pequeños animales. *Revista de Medicina Veterinaria*, (24), 59–70.

Animal Production &. Health Division, F. A. O. (2008). Pro-poor livestock policy and institutional change case studies from South Asia, the andean region and West

Africa. https://www.fao.org/publications/card/en/c/2e8db5f3-1a3b-5201-bdd6-747266487bf1/. Accessed on 02 oct 2022.
Arboine-Aguirre, L. A., Galván-García, J. E., Palacios-Rodríguez, J. M., & Guzmán-Ramírez, D. (2018). Valor pronóstico de la troponina i posintervencionismo coronario percutáneo electivo. *Gaceta Médica de México, 154*(3), 320–326.
Arphi Paccaya, F. B. (2020). *Prevalencia y pérdida económica por distomatosis en alpacas beneficiadas en la provincia de Espinar-Cusco*. Universidad Nacional de San Antonio Abad del Cusco. Bachelor's thesis.
Avilés-Esquivel, D. F., Montero, M., & Barros-Rodríguez, M. (2018). Los camélidos sudamericanos: productos y subproductos usados en la región andina. *Actas Iberoamericanas en Conservación Animal AICA, 11*, 30–38.
Becerra sánchez, J. J., del Carmen, A. O. M., & Montero Contreras, C. (2017). Diagnóstico de crianzas priorizadas para el plan ganadero 2017-2021, Lima, Peru. *Ministerio de Agricultura y Riego (MINAGRI)*, 1–67.
Blass, K. A., Kraus, M. S., Rishniw, M., Mann, S., Mitchell, L. M., & Divers, T. J. (2011). Measurement of cardiac troponin i utilizing a point of care analyzer in healthy alpacas. *Journal of Veterinary Cardiology, 13*(4), 261–266.
Cala, P. E. P., Barranco, E. A. P., Pe, S., & Bonfanti, A. C. (2015). Troponina t vs troponina i como valor predictivo para síndrome coronario agudo en menores de 80 años. *Ciencia e Innovación en Salud*, 4–10.
Canberh, B., & Kerem, U. (2018). Evaluación de biomarcadores cardiopulmonares durante diferentes etapas de leishmaniasis visceral canina. *Revista MVZ Córdoba, 23*(1), 6403–6413.
Chapman, A. R., Bularga, A., & Mills, N. L. (2020). High-sensitivity cardiac troponin can be an ally in the fight against COVID-19. *Circulation, 141*(22), 1733–1735.
Chaulin, A. M. (2021). Troponinas cardíacas: información actual sobre las principales características analíticas de los métodos para la determinación y nuevas posibilidades diagnósticas. *Medwave, 21*(11), 1–14.
Chávez, A., Leyva, V., Panez, S., Ticona, D., García, W., Pezo, D., et al. (2008). Sarcocystiosis and productive efficiency of the alpaca. *Revista de Investigaciones Veterinarias del Peru, 19*(2), 160–167.
Condori-Quispe, R., Loza-Murguía, M. G., Gutiérrez-Ramírez, L., & Condori-Condori, C. (2019). Prevalencia de sarcocystis spp. en musculo cardiaco de llamas (lama glama) y alpacas (vicugna pacos). *Journal of the Selva Andina Animal Science, 6*(2), 39–46.
Connolly, D. J., Cannata, J., Boswood, A., Archer, J., Groves, E. A., & Neiger, R. (2003). Cardiac troponin i in cats with hypertrophic cardiomyopathy. *Journal of Feline Medicine and Surgery, 5*(4), 209–216.
Contreras Flores, S. T. (2019). Potencial productivo y comercial de la alpaca. *Ministerio de Agricultura y Riego (MINAGRI)*, 1–53.
Cooper, B. J., & Valentine, B. A. (2015). Muscle and tendon. *Maxie MG. Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, 1*, 164–249.
Correa, R., & Galvis, S. (2014). Biomarcadores cardiacos como ayuda diagnóstica en las cardiopatías. *Spei Domus, 10*(20), 41–48.
Cunha, I. M., Lessa, D. A. B., Carvalho, V. A. N., Alencar, N. X., Teixeira, A. L. S., Chenard, M. G., Souza, G. N., & Helayel, M. J. (2022). Electrocardiographic, echocardiographic and heart biomarker parameters in sheep experimentally poisoned by palicourea marcgravii (rubiaceae). *Pesquisa Veterinária Brasileira, 42*, 1–9.
Cwirenbaum, R., Schmidt, A. R., Cortasa, S. A., Corso, M. C., Vitullo, A. D., Dorfman, V. B., & Halperin, J. (2021). First record of an infection by tissue cyst-forming coccidia in wild vizcachas (lagostomus maximus, rodentia) of argentina. *International Journal for Parasitology: Parasites and Wildlife, 16*, 52–58.
Dubey, J. P., Calero-Bernal, R., Rosenthal, B. M., Speer, C. A., & Fayer, R. (2015). *Sarcocystosis of animals and humans* (2nd ed.). CRC Press.
Finlayson, R., Keymer, I. F., & Manton, V. (1971). Calcific cardiomyopathy in young camels (camelus spp.). *Journal of comparative pathology, 81*(1), 71–77.
Flores Llanque, C. (2015). *Prevalencia e histopatología de sarcocistiosis cardíaca en llamas del Distrito de Conduriri, Provincia de el Collao*. Universidad Nacional del Altiplano. Bachelor's thesis.
Fraser, B. C., Anderson, D. E., White, B. J., Miesner, M. D., Wheeler, C., Amrine, D., Lakritz, J., & Overbay, T. (2013). Assessment of a commercially available point-of-care assay for the measurement of bovine cardiac troponin i concentration. *American Journal of Veterinary Research, 74*(6), 870–873.
Frenández-Baca, S. (2005). Situación actual de los camélidos sudamericanos en perú. *Proyecto de cooperación técnica en apoyo a la crianza y aprovechamiento de los camélidos sudamericanos en la región andina TCP/RLA/2914* (pp. 1–62). FAO.
Galiano, A., & Hinostroza, C. L. (2022). Producción ganadera y avícola 2021. *Anuario estadístico*. (pp. 1–164).
Guerrero, D., & Hernández, J. (1967). Ciclo evolutivo del sarcocystis. *Segundo Boletín Extraordinario IVITA*, 70–71.
Gutierrez, G. A., Gutierrez, J. P., Huanca, T., & Wurzinger, M. (2018). Challenges and opportunities of genetic improvement in alpacas and llamas in Peru. *Proceedings of the world congress on genetics applied to livestock production, Auckland, New Zealand* (pp. 12–16).
Gutiérrez, W., Vilca, F., & Calsin, B. (2015). Prevalencia e histopatología de sarcocystis lamacanis en tejido cardiaco de alpacas beneficiadas en el camal municipal de nuñoa, puno. *Libro virtual del VII congreso mundial en camélidos sudamericanos* (pp. 58–65).
Hemdon, W. E., Kittleson, M. D., Sanderson, K., Drobatz, K. J., Clifford, C. A., Gelzer, A., Summerfield, N. J., Linde, A., & Sleeper, M. M. (2002). Cardiac troponin i in feline hypertrophic cardiomyopathy. *Journal of Veterinary Internal Medicine, 16*(5), 558–564.
INIA. (2022). Sistematización de la experiencia de los subproyectos de camélidos sudamericanos financiados por el programa nacional de innovación agraria. *Ministerio de Desarrollo Agrario y Riego*.
Langhorn, R., & Willesen, J. L. (2016). Cardiac troponins in dogs and cats. *Journal of Veterinary Internal Medicine, 30*(1), 36–50.
Leguía, G., & Clavo, N. (1989). Sarcocistiosis o triquina. *Boletín Técnico N*, 5–19.
Leguía, G., & Santiago, B. (2019). Infectividad de quistes de sarcocystis sp. en alpacas (vicugna pacos) en una ganadería de la sierra central del Perú. *Biotempo, 16*(2), 209–213.
Leguía P., G., & Casas, E.. Enfermedades parasitarias y atlas parasitológico de camélidos sudamericanos1999. First ed., Edit. De Mar..
Leite, T. R. S. (2020). *Avaliação dos níveis séricos de CK-MB e troponina I em ovinos (Ovis aries) submetidos a diferentes pressões de pneumoperitônio induzido cirurgicamente*. Universidade Federal Fluminense, Faculdade de Veterinária. Master's thesis.
Li, Q., Wang, H., Zogopoulos, G., Shao, Q., Dong, K., Lv, F., Nwilati, K., Gui, X.-y., Cuggia, A., Liu, J.-L., et al. (2016). Reg proteins promote acinar-to-ductal metaplasia and act as novel diagnostic and prognostic markers in pancreatic ductal adenocarcinoma. *Oncotarget, 7*(47), 77838.
López-Torres, B., Espinoza, J., Rodríguez, J., Lucas, J., Barrios-Arpi, M., Arroyo, G., Rodríguez, A., & Revuelta, L. (2015). Correlación entre la infección por microquistes de s. lamacanis y CK-MB, AST y LDH en alpacas. *Revista Complutense de Ciencias Veterinarias, 9*(1), 40–52.
Maynard, S. J., Menown, I. B. A., & Adgey, A. A. J. (2000). Troponin t or troponin i as cardiac markers in ischaemic heart disease. *Heart, 83*(4), 371–373.
Medina, J. G. L., Leal, C. F. C., Torres, A. D. I., Salazar, A. C. H., Vázquez, J. A. A., Cuellar, J. L., & López, M. E. S. (2019). Caracterización de la curva de troponina i ultrasensible posterior al intervencionismo coronario percutáneo. *Ene, 64*(1), 6–11.
Mendes, T., Sousa, P. R., Moura, R., & Carvalho, R. (2019). Biomarcadores cardíacos e sua aplicabilidade clínica em cães e gatos. *Enciclopédia Biosfera, 16*(29).
More, G., Regensburger, C., Gos, M. L., Pardini, L., Verma, S. K., Ctibor, J., Serrano-Martínez, M. E., Dubey, J. P., & Venturini, M. C. (2016). Sarcocystis masoni, n. sp. (apicomplexa: Sarcocystidae), and redescription of sarcocystis aucheniae from llama (lama glama), guanaco (lama guanicoe) and alpaca (vicugna pacos). *Parasitology, 143*(5), 617–626.
Mostajo, W. (1983). *Sarcocistiosis en alpacas beneficiadas en el Camal Municipal de Santa Rosa, Melgar-Puno*. Universidad Nacional del Altiplano. Facultad de Medicina Veterinaria y Zootecnia.. Bachelor's thesis.
Pereira, C., Muzzi, R., Figueiredo, V. C., Muzzi, L. A. L., Oberlender, G., LJúnior, A. C. C., LNeto, M., & Oliveira, M. M. (2016). Troponina i como biomarcador de lesão cardíaca em cães com sepse. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 68*, 919–926.
Reichlin, T., Hochholzer, W., Bassetti, S., Steuer, S., Stelzig, C., Hartwiger, S., Biedert, S., Schaub, N., Buerge, C., Potocki, M., et al. (2009). Early diagnosis of myocardial infarction with sensitive cardiac troponin assays. *New England Journal of Medicine, 361*(9), 858–867.
Ministerio de Agricultura y Riego, M. I. N. A. G. R. I. (2019). Día nacional de la alpaca: Perú es potencia mundial en producción de fibra. https://www.andina.pe/agencia/noticia-dia-nacional-de-alpaca-peru-es-potencia-mundial-produccionfibra-760809.aspx. Accessed on 02 oct 2022.
Rodríguez, A., Casas, E., Luna, L., Zanabria, V., Rosadio, R., et al. (2012). Eimeriosis en crías de alpacas: prevalencia y factores de riesgo. *Revista de Investigaciones Veterinarias del Perú, 23*(3), 289–298.
Saeed, M. A., Rashid, M. H., Vaughan, J., & Jabbar, A. (2018). Sarcocystosis in south american camelids: The state of play revisited. *Parasites & Vectors, 11*(1), 1–11.
Scott, D. W., Vogel, J. W., Fleis, R. I., Miller, W. H. Jr, & Smith, M. C. (2011). Skin diseases in the alpaca (vicugna pacos): a literature review and retrospective analysis of 68 cases (cornell university 1997–2006). Veterinary dermatology, 22(1), 2–16.
Sillau, A. H., Cueva, S., Valenzuela, A., & Candela, E. (1976). O2 transport in the alpaca (lama pacos) at sea level and at 3,300 m. *Respiration Physiology, 27*(2), 147–155.
Tharwat, M. (2012). The cardiac biomarker troponin i and other haematological and biochemical variables in downer camels (camelus dromedarius). *Journal of Camel Practice and Research, 19*(2), 123–128.
Tharwat, M., Al-Sobayil, F., & Buczinski, S. (2013). Effect of racing on the serum concentrations of cardiac troponin i and creatine kinase myocardial band in racing camels (camelus dromedarius). *Veterinary research communications, 37*(2), 139–144.
Witt, K. E., & Huerta-Sánchez, E. (2019). Convergent evolution in human and domesticate adaptation to high-altitude environments. *Philosophical Transactions of the Royal Society B, 374*(1777), 20180235.