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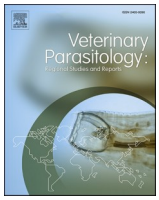

Original Article

Occurrence of paramphistomosis (Trematoda: Digenea) in sheep in northern Peru

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ABSTRACT

Cajamarca is an important Andean livestock production area located in northern Peru and at the same time endemic in a poorly studied disease known as paramphistomosis. The purpose of this research was to calculate the frequency of paramphistomids in sheep (*Ovis aries*) slaughtered in the Camal Municipal de Cajamarca, located in the city of Cajamarca. Between the months of February to September 2019, 386 samples of rumen-reticulum were collected that were later classified by age and origin of the animals. Transferred to the laboratory, the stomach contents were emptied; they were examined meticulously and exhaustively in order to find, collect and recognize the parasites adhering to the walls of the compartments. Nineteen samples were positive for *Calicophoron microbothrioides* in the rumen and none in the reticulum, thus obtaining a frequency of 4.92% in the presentation of paramphistomids in the sampled sheep. According to the origin, the town of Chanta presented a higher frequency with 9.09% and Bambamarca had the lowest frequency with 2.86%. In the age groups, sheep from 2 to 2.5 years old showed a 5.76% presence of paramphistomids and 3.33% of those 4 years old and older were positive. Pearson's correlation coefficient ($r = -0.25473762$) indicates that there is a low and inversely proportional negative relationship between age and frequency. Finally, with the Chi-square test, no significant difference was obtained between the place of origin and the number of positive cases.

1. Introduction

Domestic sheep (*Ovis aries*) are cosmopolitan animals that produce wool, meat, milk, skin, leather, and manure. Sheep are widely used by producers due to their fecundity and adaptability to new environments. They have a versatility of survival under any climate, from the coldest to the hottest, and if they have suitable climatic conditions they can produce optimally, according to their genetic potential. Due to these characteristics, sheep breeding is widespread in the Coast, Sierra and Selva of Peru. Sheep farming is economically, socially and ecologically important in these regions of Peru (Salamanca et al., 2018; van Wettere et al., 2021).

Fasciolosis is among the most important and frequent parasitic diseases found in the Peruvian Andes, causing the greatest losses in the breeding and production of sheep (Jara et al., 2018; Rodríguez-Ulloa et al., 2018). However, when coproparasitological analyzes are carried out in the laboratory, eggs of both *Fasciola hepatica* and paramphistomids are found, generating imprecise diagnoses since the forms are similar. These ruminoreticular trematodes can be the origin of many of the clinical conditions observed in sheep and of the which no serious studies are yet available in this region of Peru (Pinedo et al., 2010).

Paramphistomosis is a disease caused by digenean flukes belonging to the Paramphistomidae family. Ruminants serve as the final host and aquatic snails of the Planorbidae or Lymnaeidae families serve as an intermediate host (Müller et al., 1992; Sivajothi and Sudhakara, 2014). In Cajamarca, the presence of the intermediate host *Galba truncatula* was reported in temporarily dry environments while *Pseudosuccinea columella* is more independent of variations in its environment (Bardales-

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Valdivia et al., 2021). Paramphistomosis affects the intestines and rumen with juvenile formscausing thickening of the mucosa and submucosa of the rumen and reticulum (Bowman, 2016; Barriga, 2002). Compared to the juvenile stages, adult flukes are of little pathogenic importance, even qualifying them as "benign" (Foster et al., 2008; Mason et al., 2012). In contrast, fasciolosis affects the liver and bile ducts, causing a chronic condition that is important in Peru (Valderrama, 2016). Paramphistomids are frequently found in tropical and subtropical areas, and less frequently in temperate zones (Phiri et al., 2007). They cause great economic losses, due to low feed conversion, low productivity of infected animals and in severe cases can cause the death of the animal (Rangel-Ruiz et al., 2003).

Ovine paramphistomosis has been described in Ireland with a prevalence of 10% out of a total of 48,886 fecal samples taken from the years 2014 to 2015 (Naranjo-Lucena et al., 2018). In Jammu, province of India, a prevalence of 36.2% was reported in a total of 199 sheep (Godara et al., 2014). In Kutaber (Ethiopia) a 39.1% presence of paramphistomid eggs was evidenced in a total of 384 fecal samples (Wondmnew, 2019).

Due to the fact that this is a parasitosis of veterinary importance, very little known in Latin America and because most studies are only limited to bovines, the objective of this work was to report qualitatively for the first time the presence of paramphistomids in a significant sample of sheep slaughtered in the Camal Municipal de Cajamarca. In addition, occurrence in relation to age and place of origin was assessed.

2. Materials and methods

In the present investigation, 386 rumen-reticulum samples were collected from sheep that were of different breeds, sex, age and origin. These specimens were inspected in the Camal Municipal de Cajamarca between the months of February to September 2019. The animals came from six distant places in the Cajamarca region, which are higher than 2000 m above sea level and have two very marked weather seasons: the rainy season (October to April) and the dry season (May to September). The protocol used for slaughter at the Camal Municipal de Cajamarca is governed by the Reglamento Sanitario del Faenado de Animales de Abasto (Servicio Nacional de Sanidad Agraria, 2012).

At the abattoir, 44 head of sheep on average are slaughtered daily. A 10% sampling rate was selected with collection occuring three times a week, on Monday, Wednesday and Friday. At each visit, 4 or 5 sheep were inspected.

Once the sheep had been randomly selected, the rumen and reticulum were collected after slaughter and separated in plastic buckets. The pillars (anterior and posterior) of the rumen-reticulum were observed and only adult parasites adhered to the mucosa of these areas were searched for collection for identification.

For the correct identification of the paramphistomids, first, the parasites were visually recognized in the rumen and then they were observed in a stereoscope in the Laboratory of Veterinary Parasitology of the Universidad Nacional de Cajamarca (Fig. 1). There, the morphological characteristics exhibited by the collected parasites and which are detailed in other reports were taken into account (Eduardo, 1937; Sey, 1982). For taxonomic identification, the Eduardo (1982, 1985) and Jones et al. (2005) keys were used.

Once the data from the observational analysis had been obtained, they were grouped by variables (age and origin) to express their frequency.

Descriptive statistics were used in Microsoft Excel software, making frequency tables, charts and graphs. Also, Pearson's correlation analysis was performed to know the relationship between the age of the animals and the frequency of paramphistomosis. Finally, the Chi-square test was performed to find out whether or not there was a significant difference between the places and the number of cases presented in each one.

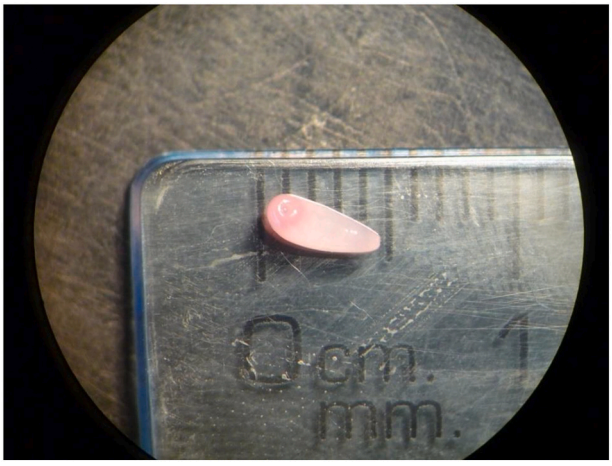

Fig. 1. Paramphistomid observed with a stereoscope with a measurement of 0.5 cm.

3. Results

In total, 19 sheep (4.92%) were infected with Paramphistomids and the parasites were only found in the rumen (Table 1, Fig. 2). The trematodes had an average length of 0.58 cm and were described as *Calicophoron microbothrioides* according to the taxonomic keys used that allowed their characterization (Fig. 3). Regarding the place of origin, the town of Chanta showed a higher frequency with 9.09% ($n = 22$) and Bambamarca obtained the lowest frequency with 2.86% ($n = 70$).

As Table 2 shows, the age group with the highest frequency of paramphistomosis is between the age of "two teeth" (5.45%) and "four teeth" (5.76%), that is, a year to three years approximately.

When statistical analysis was performed using Pearson's correlation coefficient, the value $r = -0.25473762$ was obtained, indicating that there is an inversely proportional relationship for age and frequency of paramphistomosis. This means that, as the age increases, the frequency decreases; even so, due to the value found, there is a low negative correlation.

In the Chi-square test, it was determined that $p = 0.828888$; therefore, $p > 0.05$ and there is no significant difference between the places where the animals come from with the number of positive cases presented to paramphistomids. When performing the Shapiro-Wilk normality test, working with the monthly averages of positive cases of paramphistomosis according to the place of origin, a uniform distribution was found in the data during the eight months of sampling, there were no statistically significant variations.

4. Discussion

The frequency of paraphistomosis in sheep found for Cajamarca (4.92%) cannot be compared with local results since there is no record of

Table 1
Frequency of paramphistomosis in sheep inspected in the Camal Municipal de Cajamarca, according to origin (February–September 2019).

Origin of the Sheep	Number of Animals Inspected	Frequency			
		Negative		Positive	
		N°	%	N°	%
Cajamarca	170	165	94.12	10	5.88
Jesús	85	82	96.47	3	3.53
Bambamarca	70	68	97.14	2	2.86
Chanta	22	20	90.91	2	9.09
San Marcos	20	14	95.00	1	5.00
La Encañada	19	18	94.74	1	5.26
TOTAL	**386**	**367**	**95.08**	**19**	**4.92**

Fig. 2. Rumen of sheep with the presence of two paramphistomids adhered to the mucosa (black arrows).

Fig. 3. Paramphistomidae in dark background obtained in the Camal Municipal de Cajamarca.

Table 2
Frequency of presentation of paramphistomosis in sheep inspected in the Camal Municipal de Cajamarca, according to age (February–September 2019).

Age	Number of Animals Sampled	Frequency			
		Negative		Positive	
		N°	%	N°	%
Milk teeth (0–1 year old)	57	55	96.49	2	3.51
Two teeth (1–1.5 years old)	110	104	94.55	6	5.45
Four teeth (2–2.5 years old)	139	131	94.24	8	5.76
Six teeth (3–3.5 years old)	50	48	96.00	2	4.00
Full mouth (4 or more years old)	30	29	96.67	1	3.33
TOTAL	**386**	**367**	**95.08**	**19**	**4.92**

such information; however, Vasquez and Torrel (2007), found a prevalence of 5.07% in dairy cattle from Cajamarca, their findings being very similar to ours. Furthermore, more than a decade ago it was reported that *Calicophoron microbothrioides* was the only bovine paramphistomid found, described with the same taxonomic keys used in the present study; however, we emphasize that molecular techniques must be used for its confirmation (Ortiz et al., 2010). (Ortiz et al., 2010).

A study in Ireland indicates average prevalences of 10% between 2014 and 2015 in cattle and sheep (Naranjo-Lucena et al., 2018). In India, the prevalence in sheep and goats was 36.2%, approximately six times that of our results. (Godara et al., 2014). A similar phenomenon occurs in Ethiopia, where the findings reach a high prevalence of 39.1% according to the stool tests performed there (Wondmnew, 2019).

The highest frequency is found in animals from the town of Chanta with 9.09%, probably due to the precarious management of crops with traditional flood irrigation systems, the extensive rearing of herds and which are predominant characteristics in the Peruvian Andes; all this allowing the optimal development of the biological cycle of the paraphystomids and favoring that the animals become infected and reinfected periodically (Salamanca et al., 2018). Due to the paraphistomosis frequency values found in each place of origin, the Chi-square test indicates that there is no influence between the area and the presentation of positive cases; Therefore, the participation of the environment in the development of the biological cycle of the parasite is uniform in the areas of origin of the sampled animals. It is appropriate to carry out a study where more ecological variables are considered and that allow clarifying the behavior of paramphistomids in various geographical levels of Peru.

Future research on the association of paramphistomids with *Fasciola hepatica* could be useful since Rasco (2007) identified mixed infections with a prevalence of 25.20% for cattle. This could also be happening in sheep.

A very notable difference lies in the methodology used, because the aforementioned investigations use coproparasitological analysis and we use necropsy. Greater dissemination of diagnostic techniques for this trematode is needed, including molecular tests such as polymerase chain reaction, which will even allow the detection of parasites in the reticulum, which is little studied at necropsy, and in juvenile stages present in the intestine (Martinez-Ibeas et al., 2016).

5. Conclusion

In South America, paramphistomids have not been rigorously studied in small ruminants. For the first time in Peru, *Calicophoron microbothrioides* is described as the species that affects sheep and some risk factors associated with infection are detailed. The prevalences found are low; Even so, more research is needed to understand the role of sheep in the spread of the parasite and also the lethality that it could cause in different animal species of veterinary importance (cattle, sheep and

goats), added in many cases to the coexistence with *F. hepatica* and that should be fully controlled because they share the same intermediate host.

Funding

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Ethical statement

No direct animal testing. The samples were collected from sheep slaughtered in a Municipal Slaughterhouse inspected by a Veterinary Doctor and where welfare is ensured according to Peruvian regulations.

Declaration of Competing Interest

None.

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