

DOI: https://dx.doi.org/10.54203/scil.2025.wvj98 PII: S232245682500098-15

# Prevalence and Parasite Load of Helminths in Reintroduced Vicuñas (*Vicugna vicugna*) in High-Andean Communities, Huancavelica, Peru

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#### ABSTRACT

Gastrointestinal helminth infections represent a major threat to the health and conservation of vicuñas (Vicugna vicugna) in the Peruvian Andes, while epidemiological information on repopulated populations under communitybased management remains limited. The present study aimed to determine the prevalence and parasite load of gastrointestinal helminths in reintroduced vicuñas from the high-Andean communities of Huancavelica, Peru. In the present study, 102 vicuñas from the communities of Atuna and Allarpo (Huancavelica), Peru, were evaluated during the 2023 chaccu, a traditional Andean practice in which wild vicuñas are collectively herded, captured, and shorn for fiber under community supervision. The sample consisted of 66 females and 36 males, of which 80 were classified as adults (>3 years old) and 22 as juveniles (1-3 years old). Rectal fecal samples were collected and analyzed to detect and quantify gastrointestinal helminth eggs, including strongyle-type eggs, Nematodirus, Trichuris, and Fasciola hepatica (F. hepatica). Samples were analyzed using flotation, modified McMaster, and sedimentation techniques to detect and quantify helminth eggs. Overall prevalence, including by community, sex, age, and parasite load (eggs per gram, EFG) as a geometric mean, was determined. The overall prevalence was 75.5%, with F. hepatica as the most frequent parasite, 79.2% in Allarpo and 60.9% in Atuna, followed by Trichuris spp. (11.6%) and Nematodirus spp. (10.2%), and Strongyle-type eggs 42.0% in Allarpo and 17.45% in Atuna. Juveniles had a higher prevalence of F. hepatica infection (90.9%) compared to adults (71.3%). The mean F. hepatica egg count was higher in Allarpo (34.4 EPG) than in Atuna (11.7 EPG), and this difference was statistically significant. When analyzed by sex, females indicated a higher mean egg count (24.6 EPG; 95% CI: 16.9-35.7) than males (17.4 EPG; 95% CI: 9.8-30.7). Regarding age, adults presented a higher mean EPG (23.6; 95% CI: 16.0-34.8) compared to juveniles (16.5; 95% CI: 8.8-31.1), although mentioned differences were not statistically significant. The findings of the present study indicated that helminth infections, particularly fascioliasis, are widespread, with higher risk in juveniles and environmentally favorable areas, highlighting the need for locally adapted monitoring and control strategies in reintroduced vicuña populations.

Keywords: Fasciola hepatica, Gastrointestinal helminth, Prevalence, Vicugna vicugna

### INTRODUCTION

South American camelids play a fundamental role in the livelihoods of high-Andean populations, representing a genetic resource of high economic, social, cultural, and scientific value (González et al., 2019; Vilá and Arzamendia, 2022). Among them, the vicuña (Vicugna vicugna) is a wild species highly prized for the exceptional quality of its fiber, considered one of the most valuable natural fibers worldwide. However, gastrointestinal helminthiasis can negatively affect body condition, metabolism, and consequently fiber production and quality, thereby reducing the economic benefits derived from community-based management programs such as the chaccu. Understanding the epidemiology of these infections is therefore essential to ensure both animal health and the sustainable use of this valuable resource. Peru is the leading exporter of this fiber, which can reach prices of up to USD 400/kg in the international market, mainly destined for the luxury fashion industry (Quispe et al., 2009). Due to conservation programs and sustainable management, populations that had been decimated by overexploitation have recovered significantly. In several high-Andean regions, this process has included repopulation initiatives, involving the reintroduction of vicuñas into areas where their presence was scarce or absent (Lichtenstein 2009; Wheeler and Hoces 1997). vicuñas are translocated from zones with stable wild populations to high-Andean areas where the species had previously declined. The translocation is coordinated by the National Forest and Wildlife Service (SERFOR) and implemented by local communities under

Received: October 07, 2025
Revised: November 04, 2025
Accepted: December 05, 2025
Published: December 25, 2025

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sustainable management programs. Once established, these populations are managed through annual chaccu events-traditional roundups organized for health monitoring and fiber shearing under official supervision. While these actions have strengthened species conservation and generated local economic benefits, they also pose health challenges, as management conditions, contact with domestic livestock, and adaptation to new environments can increase exposure to pathogens, particularly gastrointestinal helminths (Pérez et al., 2014; Angulo, 2021).

Gastrointestinal helminths infections negatively affect body condition, survival of young vicuñas, and fiber quality. Previous studies in wild and semi-captive vicuñas have reported prevalences of up to 80% for different genera of nematodes and trematodes (Marcoppido et al., 2016; Morsy et al., 2020), with *Fasciola hepatica* (*F. hepatica*) being one of the most important veterinary and zoonotic species in the Andean region of Peru. Transmission is influenced by environmental factors such as altitude, humidity, and the presence of intermediate hosts; by management practices including mixed grazing with domestic livestock, and by host-related factors such as age and immune status (Londoñe et al., 2009; Ibrahim, 2017). In high-Andean communities such as Allarpo-Chillama and Atuna-Quihuay in the Huancavelica region, Peru, the high prevalence of gastrointestinal helminths may be exacerbated by the inadequate use of anthelmintics, close contact with domestic livestock, and the effects of climate change, which can also contribute to the development of resistance to conventional treatments (Cafrune et al., 1996; Kelley et al., 2016; Chugá and Martínez, 2024).

Given the limited epidemiological information available for repopulated vicuñas under community-based management, this study aimed to determine the prevalence and parasite load of gastrointestinal helminth infections in vicuñas from the rural communities of Allarpo-Chillama and Atuna-Quihuay (Huancavelica, Peru).

### MATERIALS AND METHODS

#### **Ethical approval**

Sample collection was carried out exclusively on vicuñas captured as part of the routine chaccu process, authorized and supervised by the National Forest and Wildlife Service (SERFOR). No additional capture or handling was performed for experimental purposes beyond the procedures established by the competent authorities. All activities complied with the animal welfare principles established by Peruvian Law No. 30407 on Animal Protection and Welfare.

# Study area

The study was conducted in the rural communities of Allarpo-Chillama and Atuna-Quihuay, located in the Huancavelica region, Peru. These communities are located in the district of Seclla, Angaraes Province, in the Huancavelica region of Peru (approximately -13.05° S, -74.48° W). They are situated about 26 km east-southeast of the regional capital city of Huancavelica (-12.79° S, -74.98° W). The study sites are located at altitudes of 4,100 and 4,200 m above sea level, respectively, with a mean annual temperature of 10.7°C and an average relative humidity of approximately 65%. Local economic activities included livestock farming, subsistence agriculture, and vicuña management for fiber production. The study area covered approximately 60 km² and included vicuña repopulation zones selected for conservation programs of the species. The geographical location of the sampling sites, along with their spatial distribution within the Secclla district, is illustrated in Figure 1.



Figure 1. Location of the vicuña sampling sites in the communities of Allarpo (A) and Atuna (B), Secolla district, Angaraes province, Huancavelica, Peru. Markers (red) indicate the approximate capture areas where fecal samples were collected during the chaccu. The inset map showed the position of the Huancavelica region within Peru.

## Study design and animals

Sampling strategy and sample size were determined by the legal and operational constraints of working with protected wildlife. Because vicuñas are a protected species and captures are strictly restricted to the annual chaccu authorized by SERFOR, no a priori sample size calculation was performed. Instead, all individuals safely captured during the roundup were sampled, yielding a pragmatic census of the captured subset (n = 102). The resulting sex ratio reflects the natural social structure of vicuña family groups and the operational constraints of the capture, so equal numbers by sex could not be enforced. Vicuñas were classified as adults (> 3 years) or juveniles (1-3 years) based on field estimates of body size, dentition, and general morphology, since individual age records were not available. The agebased classification method is commonly applied during chaccu events, where handling time is limited, and animals are released immediately after sampling. The evaluated individuals originated from an original population translocated from the district of Pilpichaca, Huaytará, Huancavelica. Capture and handling were carried out as part of the official management authorized by the Servicio Nacional Forestal y de Fauna Silvestre (SERFOR), a public agency under the Ministry of Agrarian Development and Irrigation (MIDAGRI) of Peru.

## Sample collection and parasitological analysis

Fecal samples were obtained directly from the rectum of each vicuña, between 11:00 a.m. and 14:00 p.m., using new sterile disposable latex gloves for each individual to avoid cross-contamination and ensure biosafety. Each sample was placed in a hermetically sealed bag labeled with individual animal data, including age, sex, community of origin, and sampling date. A minimum of 15 g of feces was collected per animal for laboratory analysis. Samples were kept at 4° C in polystyrene containers for transport to the Microbiology and Parasitology Laboratory, Parasitology Section, Faculty of Veterinary Medicine, National University of San Marcos (UNMSM), in San Borja, Lima, Peru, following recommended procedures for fecal sample collection and transport in parasitological study (Taylor et al., 2007).

### Parasitological analysis methods

#### Qualitative flotation technique with a supersaturated sugar solution)

A 1-2 g fecal sample was homogenized in 20 mL of water, filtered, and allowed to sediment. The sediment was resuspended in sugar solution to form a meniscus, covered with a coverslip, allowed to stand for 10 min, and examined under a microscope (10X; Leica Microsystems DM 750, Germany) to identify parasitic forms. This flotation method, based on differences in specific gravity, enables the recovery of light nematode and cestode eggs from fecal suspensions (Ministerio de Salud, 2002; Taylor et al., 2007).

## Modified McMaster quantitative technique

The modified McMaster technique was used to detect and quantify oocysts, nematode eggs, and cestode eggs present in fecal samples. Approximately 3 g of feces were weighed, disaggregated in a mortar, and diluted with 42 mL of water. The mixture was filtered through a fine mesh sieve of 200  $\mu$ m aperture into a test tube and allowed to stand for 30 minutes, or alternatively centrifuged at 1,500 rpm for 3 minutes. After discarding the supernatant, the sediment was homogenized with saturated saline solution to a final volume of 10 mL (Taylor et al., 2007; Bondarenko et al., 2009). Using a Pasteur pipette, both chambers of the McMaster slide (total volume of 1 mL) were filled, and the preparation was examined under a microscope (10X; Leica Microsystems DM 750, Germany) for parasite counting. The calculation of parasite load, expressed as eggs per gram (EPG), was based on the total volume of the fecal suspension examined under the microscope. When only two grids of the McMaster chamber (equivalent to 0.3 mL) were observed, the following formula was applied, EPG = A × 50. When the entire chamber (1 mL) was read, the following formula was used; EPG = A × 15 (A represents the number of parasitic forms counted). This volume-based adjustment ensures that the estimated EPG accurately reflects the proportion of eggs observed in relation to the examined volume. The methodological parameters were standardized according to previously validated protocols and adapted field guidelines (University of Rhode Island, 2018).

#### Dennis sedimentation method

The sedimentation method was performed according to the procedures described by Rojas-Moncada et al. (2024). This technique is used to concentrate and observe parasite eggs present in fecal samples. The procedure involved weighing between 3 and 6 g of feces, placing them in a mortar (Cole-Parmer, USA), and mixing with a 1% detergent solution. The sample is filtered into a 250 mL sedimentation cup, allowed to settle for 10-12 minutes, and the supernatant is decanted. The resuspension-sedimentation procedure using a 1% detergent solution was repeated two additional times. The process of resuspension and sedimentation with 1% detergent solution was repeated two additional

times to improve sediment cleaning. Subsequently, 4 to 6 drops of Lugol's iodine (Sigma-Aldrich, Merck, Germany) are added and allowed to act for 5 minutes to stain the sediment material.

The stained sediment is emulsified with running water and divided into several test tubes, which are centrifuged (Hettich, Germany) 1,500 rpm for 3 minutes. The resulting sediments are combined into a single tube and centrifuged again under the same conditions. Finally, the sediment is resuspended in 10 mL of water, and using a gridded Petri dish, the eggs present in the entire volume are counted. To express the parasite load as eggs per gram of feces (EPG), the total number of eggs recovered in the Petri dish was divided by the exact weight of feces used in the assay, following the standard formula, EPG = Total eggs counted / grams of feces processed. The Dennis sedimentation method allowed for clearer and more precise observation of parasites by concentrating and staining them, thereby facilitating their identification and quantification.

The three complementary parasitological techniques were employed to maximize the detection of a broad spectrum of helminth eggs and protozoan oocysts. The flotation method allowed for the qualitative identification of light nematode and cestode eggs, the modified McMaster technique provided a quantitative estimation of infection intensity, and the sedimentation method enables the detection of heavy trematode eggs such as *Fasciola hepatica* (Foreyt, 2001; Taylor et al., 2007).

#### Justification of quantitative indicators

The prevalence of helminth infections was calculated as a percentage, differentiating results by community of origin, sex, and age group of the evaluated vicuñas. Parasite load, EPG, indicated an asymmetric distribution with dispersed values. Therefore, the geometric mean (GM) was used as the measure of central tendency. The use of geometric mean provided a more accurate representation of parasitological data, as counts obtained through the McMaster and Dennis techniques typically indicate skewed distributions, with most values in the lower range and a few extremely high counts. Such irregularity can distort the arithmetic mean, overestimating the actual value. The GM, in contrast, offers a more representative estimate of the overall infection level in the evaluated population by minimizing the influence of extreme values (Ojja et al., 2018).

### Statistical analysis

Data were organized and processed using R statistical software version 4.4.3 (R Core Team, 2025). To assess the relationship between the presence or absence of helminths and categorical variables such as sex, age, and community, the Chi-square test was applied. To compare parasite load between groups (males versus females, juveniles versus adults), the Mann-Whitney U test was used, as it is a non-parametric method suitable for non-normally distributed data (Nahm, 2016). A significance level of p < 0.05 was considered for all analyses, with p-values obtained from Chi-square tests (for prevalence comparisons) and Mann-Whitney U tests (for comparisons of parasite load).

# **RESULTS**

The consolidated analysis of helminthiasis in vicuñas (*Vicugna vicugna*) from the communities of Allarpo and Atuna, Huancavelica, Peru, revealed a high overall infection frequency of 75.5% (Table 1). When results were disaggregated by sex, males indicated a slightly higher infection frequency (80.6%) compared to females (72.7%). Although the numbers of males and females examined were not equivalent, the comparison remains statistically sound because prevalence differences were evaluated with a Chi-square test, which accommodates unequal group sizes. The calculated odds ratio (OR = 1.55) indicated a higher likelihood of infection in males; however, this association was not statistically significant (p > 0.05; 95% CI: 0.58-4.17).

Regarding the age groups, juveniles (1-3 years) showed a markedly higher infection frequency (90.9%) compared with adults (> 3 years; 71.3%). However, because the juvenile group was smaller in size, this difference should be interpreted as an epidemiological trend rather than a statistically confirmed effect. Although the odds ratio indicated lower odds of infection in adults (OR = 0.25), the association did not reach statistical significance (p > 0.05; 95% CI: 0.054-1.15). The overall frequency of helminths in the sampled vicuñas was 69.2% in Atuna (Table 2) and 82.0% in Allarpo (Table 3). In Atuna, juveniles showed a higher infection frequency (91.7%) than adults (62.5%), corresponding to an odds ratio of 6.60. This OR indicates that juveniles were more likely to be infected; however, the association was not statistically significant (p > 0.05), and therefore represents an epidemiological trend rather than a confirmed difference. The unequal distribution of vicuñas across age classes reflects the natural demographic structure observed during the chaccu, and because all safely captured animals were sampled, the resulting dataset still provides a reliable epidemiological baseline. In Allarpo, a similar pattern was observed, with juveniles presenting a higher infection frequency (90.0%) than adults (80.0%), although this association was again not statistically significant (OR = 2.25; p > 0.05).

Table 1. Frequency of helminths in vicuñas by sex and age from Allarpo and Atuna districts in Huancavelica, Peru

| Variable        | Number of vicuñas | N  | %    | OR   | P value | CI           |
|-----------------|-------------------|----|------|------|---------|--------------|
| Sex             |                   |    |      |      |         |              |
| Female          | 66                | 48 | 72.7 | 0.65 | -       | -            |
| Male            | 36                | 29 | 80.6 | 1.55 | 0.47    | 0.58 - 4.17  |
| Age (years)     |                   |    |      |      |         |              |
| Adults (> 3)    | 80                | 57 | 71.3 | 0.25 | 0.091   | 0.054 - 1.15 |
| Juveniles (1-3) | 22                | 20 | 90.9 | 4.00 | -       | -            |
| Total           | 102               | 77 | 75.5 |      |         |              |

Note: N: Positive cases, OR: Odds ratio, P: Statistical significance level, CI: Confidence interval

Table 2. Frequency of helminths in vicuñas by sex and age in Atuna, Huancavelica, Peru

| Variable        | Number of vicuñas | N  | %    | OR   | P value | CI           |
|-----------------|-------------------|----|------|------|---------|--------------|
| Sex             |                   |    |      |      |         |              |
| Female          | 35                | 23 | 65.7 | 0.59 | -       | -            |
| Male            | 17                | 13 | 76.5 | 1.70 | 0.532   | 0.45 - 6.35  |
| Age (years)     |                   |    |      |      |         |              |
| Adults (> 3)    | 40                | 25 | 62.5 | 0.15 | 0.078   | 0.018 - 1.29 |
| Juveniles (1-3) | 12                | 11 | 91.7 | 6.60 | -       |              |
| Total           | 52                | 36 | 69.2 |      |         |              |

Note: N: Positive cases, OR: Odds ratio, P: Statistical significance level, CI: Confidence interval

Table 3. Frequency of helminths in vicuñas by sex and age in Allarpo, Huancavelica, Peru

| Variable        | Number of vicuñas | N  | %    | OR   | P value | CI          |
|-----------------|-------------------|----|------|------|---------|-------------|
| Sex             |                   |    |      |      |         |             |
| Female          | 31                | 25 | 80.6 | _    | -       | -           |
| Male            | 19                | 16 | 84.2 | 0.78 | 0.9     | 0.17 - 3.58 |
| Age (years)     |                   |    |      |      |         |             |
| Adults (> 3)    | 40                | 32 | 80.0 | _    | 0.665   | 0.05 - 4.04 |
| Juveniles (1-3) | 10                | 09 | 90.0 | 2.25 | -       | -           |
| Total           | 50                | 41 | 82.0 | -    | -       | -           |

Note: N: Positive cases, OR: Odds ratio, P: Statistical significance level, CI: Confidence interval

In Atuna, Strongyle-type eggs (STE) were the most prevalent (17.45%), followed by *Trichuris* spp. (11.6%) and *Nematodirus* spp. (10.2%; Table 4). When comparing parasite species between adults and juveniles, the highest frequency was recorded for *F. hepatica* (60.9%) in Atuna. Within this community, prevalence was significantly higher in juveniles (91.7%) than in adults (50.0%; p < 0.05). In Allarpo, the frequency of STE was higher (42.0%), while *F. hepatica* reached 79.2%. Juveniles indicated a higher prevalence (90.0%) than adults (75.0%); however, no statistically significant difference was observed (Table 5).

In Table 6, the GM of the STE load was higher in Allarpo (69.5 EPG; 95% CI: 54.7-88.3) than in Atuna (50.2 EPG; 95% CI: 49.9-50.6), although the difference was not statistically significant (p > 0.05). The GM was used because it is less sensitive to extreme values compared to the arithmetic mean. For *F. hepatica*, the GM egg count was significantly higher in Allarpo (34.4 EPG; 95% CI: 22.5-52.6) than in Atuna (11.7 EPG; 95% CI: 7.5-18.2; p < 0.05). At the overall study level, females showed a higher *Fasciola hepatica* egg load (24.6 EPG; 95% CI: 16.9-35.7) compared with males (17.4 EPG; 95% CI: 9.8-30.7), although this difference was not statistically significant (p > 0.05). The GM of STE in males was 64.0 EPG (95% CI: 44.4-92.3), slightly higher than in females, 58.4 EPG (95% CI: 49.8-68.5). These values represent egg counts per gram (EPG), not percentages. According to age, adults indicated a higher *Fasciola hepatica* egg load 23.6 EPG (95% CI: 16.0-34.8) than juveniles 16.5 EPG (95% CI: 8.8-31.1); however, this difference was not statistically significant (p > 0.05). As indicated in Table 6, no superscript letters appear in this comparison, confirming the absence of statistical significance between age groups.

For *Nematodirus* spp. and *Trichuris* spp., the geometric mean (GM) and 95% CI appear as 50.0 (50.0-50.0) in several categories. This uniform value reflects the minimum detection threshold of the modified McMaster technique used in this study (1 egg = 50 EPG), and indicates that all positive animals for these parasites had counts at or near this lower detection limit rather than identical biological loads. Skrjabinema spp. was not included in the geometric mean (GM) analysis because the number of positive animals was insufficient to calculate a reliable GM value using the McMaster method.

No significant differences in STE load were observed between males and females (p > 0.05) or between adults and juveniles (p > 0.05). The same pattern was found for the other parasites. The only statistically significant difference corresponded to *Fasciola hepatica*, whose load was higher in Allarpo than in Atuna (p < 0.05).

Table 4. Prevalence of helminth eggs in vicuñas by sex and age in Atuna district, Huancavelica, Peru

| Variable        | Number of vicuñas | Strongyle-type<br>eggs (%) | Nematodirus<br>(%) | Trichuris (%)     | Skrjabinema<br>(%) | F. hepatica (%)     |
|-----------------|-------------------|----------------------------|--------------------|-------------------|--------------------|---------------------|
| Sex             |                   |                            |                    |                   |                    |                     |
| Female          | 35                | 11.4 <sup>b</sup>          | $8.6^{\mathrm{b}}$ | 5.7 <sup>b</sup>  | $2.9^{b}$          | 57.1 <sup>a</sup>   |
| Male            | 17                | 23.5 b                     | 11.8 <sup>b</sup>  | 17.6 <sup>b</sup> | 5.8 <sup>b</sup>   | 64.7 <sup>a</sup>   |
| Age (years)     |                   |                            |                    |                   |                    |                     |
| Adults (>3)     | 40                | 12.5 b,B                   | 10.0 b,B           | 7.5 b,B           | 2.5 b,B            | 50.0 <sup>a,B</sup> |
| Juveniles (1-3) | 12                | 25.0 b,B                   | 8.3 b,B            | 16.7 b,B          | 8.3 b,B            | 91.7 <sup>a,A</sup> |
| Total           | 52                | 17.5                       | 10.2               | 11.6              | 4.3                | 60.9                |

Note: Different superscript lowercase letters (a-b) within each row indicate a statistically significant difference (p < 0.05). Different superscript uppercase letters ( $^{A,B}$ ) within each column indicate a statistically significant difference (p < 0.05).

Table 5. Prevalence of helminth eggs in vicuñas by sex and age in Allarpo district, Huancavelica, Peru

| Variable        | Number of vicuñas | Strongyle-type<br>eggs (%) | Nematodirus<br>(%) | Trichuris<br>(%)  | Skrjabinema<br>(%) | F. hepatica (%)     |
|-----------------|-------------------|----------------------------|--------------------|-------------------|--------------------|---------------------|
| Sex             |                   |                            |                    |                   |                    |                     |
| Female          | 31                | 41.9 <sup>b</sup>          | 19.4 <sup>b</sup>  | 6.5 °             | 3.2 °              | 74.2 <sup>a</sup>   |
| Male            | 19                | 42.1 <sup>b</sup>          | 21.1 <sup>b</sup>  | 15.8 <sup>b</sup> | 0                  | 84.2 <sup>a</sup>   |
| Age (years)     |                   |                            |                    |                   |                    |                     |
| Adults (>3)     | 40                | 42.5 b,B                   | 20.0 c,B           | 5.0 d,B           | 2.5 <sup>d</sup>   | 75.0 <sup>a,B</sup> |
| Juveniles (1-3) | 10                | 40.0 b,B                   | 20.0 b,B           | 30.0 b,A          | 0                  | 90.0 <sup>a,B</sup> |
| Total           | 50                | 42.0                       | 20.3               | 11.2              | 3.2                | 79.2                |

Note: Different superscript lowercase letters ( $^{a\cdot b}$ ) within each row indicate a statistically significant difference (p < 0.05). Different superscript uppercase letters ( $^{A,B}$ ) within each column indicate a statistically significant difference (p < 0.05).

**Table 6.** Geometric mean of parasite load of Strongyle-type eggs, Nematodirus spp., *Trichuris* spp., and *Fasciola hepatica* spp. present in positive vicuñas from Atuna and Allarpo districts in Huancavelica, Peru

| \$7 <b>!</b> - 1-1 - | Strongyle-type eggs (STE) | Nematodirus | Trichuris   | F. hepatica       |  |
|----------------------|---------------------------|-------------|-------------|-------------------|--|
| Variable             | GM (CI)                   | GM (CI)     | GM (CI)     | GM (CI)           |  |
| Sex                  |                           |             |             |                   |  |
| г 1                  | 58.4                      | 50.0        | 50.75       | 24.6              |  |
| Female               | (49.8-68.5)               | (50.0-50.0) | (50.3-51.2) | (16.9-35.7)       |  |
| M-1-                 | 64.0                      | 50.0        | 57.2        | 17.4              |  |
| Male                 | (44.4-92.3)               | (50.0-50.0) | (45.7-71.4) | (9.8-30.7)        |  |
| Age (years)          |                           |             |             |                   |  |
| Adults (>3)          | 64.1                      | 50.0        | 58.2        | 23.6              |  |
|                      | (44.5-92.4)               | (50.0-50.0) | (44.5-76.3) | (16.0-34.8)       |  |
| Juveniles (1-3)      | 61.3                      | 50.0        | 50.6        | 16.5              |  |
|                      | (47.6-78.9)               | (50.0-50.0) | (50.1-51.1) | (8.8-31.1)        |  |
| )rigin               |                           |             |             |                   |  |
| Atuna                | 50.2                      | 50.0        | 50.8        | 11.7              |  |
|                      | (49.9-50.6)               | (50.0-50.0) | (50.4-51.2) | (7.5-18.2)        |  |
| Allarpo              | 69.5                      | 50.0        | 58.0        | 34.4 <sup>a</sup> |  |
|                      | (54.7-88.3)               | (50.0-50.0) | (44.2-76.1) | (22.5-52.6)       |  |
| Total (CI 95%)       | 63.6                      | 50.0        | 54.3        | 21.3              |  |
|                      | (53.1-76.1)               | (50.0-50.0) | (47.4-62.2) | (15.3-29.7)       |  |

Note: 95% CI; a p = 0.04 (Mann–Whitney U test), CI: confidence interval.

#### DISCUSSION

The present study revealed a high overall prevalence of gastrointestinal helminths (75.5%) in vicuñas evaluated during the 2023 Chaccu in the communities of Allarpo and Atuna, confirming the persistent presence of gastrointestinal parasites in semi-captive management systems. The observed pattern is consistent with other reports from high Andean areas, where predisposing factors such as communal grazing, lack of systematic sanitary control, and favorable

ecological conditions contribute to helminth transmission (Marcoppido et al., 2016; Curay et al., 2022). In the present study, parasite detection was based on coprological examination, a method widely used in field surveys to estimate infection levels and describe general patterns of helminth occurrence. While this approach allows reliable assessment of prevalence and parasite burden in wildlife populations, more detailed identification would require additional laboratory techniques. Similar levels of gastrointestinal helminth infections have been described in other high-Andean camelid populations, such as alpacas from Macusani, Puno, where high prevalences were observed even during the dry season (Contreras et al., 2014). The results support the idea that helminth circulation remains consistently high in communal pastoral systems across the puna.

Among all the parasites detected, *Fasciola hepatica* clearly stood out. Its prevalence was higher in both communities and particularly marked in Allarpo (79.2%). The environmental conditions in this area—greater humidity, wetlands, and waterlogged soils, likely favor the presence and survival of Lymnaea spp., the intermediate host essential for the parasite's life cycle (Mas-Coma et al., 2009). Other environmental drivers such as altitude, temperature, vegetation structure, and water availability have been widely linked to fascioliasis transmission in Andean landscapes (Flores et al., 2014; Charlier et al., 2020). The significantly higher egg load recorded in Allarpo reinforces the idea that local ecological conditions shape infection intensity. The presence of domestic livestock, especially sheep and cattle, may add an additional layer of risk by increasing environmental contamination with infective stages (Julón et al., 2020; Angulo et al., 2021).

The overall prevalence of helminths was 69.2% in Atuna and 82.0% in Allarpo, similar to the  $81.3\% \pm 5.3\%$  reported in Cajamarca (Curay et al., 2022). This difference may be explained by variations in altitude, climatic conditions, and the level of interaction with other livestock species that serve as parasite reservoirs. Altitude and climatic conditions are known to significantly influence the epidemiology of helminth infections (Aguirre and Cafrune, 2007; Mas-Coma et al., 2009). Higher altitude and lower temperatures can affect the development and survival of larval stages in the environment. Temperatures around  $15^{\circ}$ C and a minimum monthly precipitation of 50 mm are ideal for the survival of many helminth larvae (Charlier et al., 2020). Additionally, some parasite eggs have been observed to withstand freezing temperatures as low as  $-16^{\circ}$ C for extended periods, allowing their development in high altitude environments.

Increases in temperature can enhance parasite reproduction rates, altering helminth transmission dynamics (Rodríguez et al., 2013; Samamé et al., 2016; Chugá and Martínez, 2024). Previous studies in South American camelids have reported variations in helminth prevalence depending on water availability and pasture type (Issia, 2009), finding that vicuñas with greater access to water bodies have higher infections by trematodes such as *F. hepatica*. This is consistent with the present findings, where Allarpo, a more humid community, indicated a significantly higher prevalence of *F. hepatica*. Interaction with domestic livestock also plays a key role in helminth transmission to vicuñas. A study in llama and alpaca populations (Angulo et al., 2021), found that those sharing grazing areas with sheep and cattle had higher parasite loads, particularly gastrointestinal nematodes. The coexistence of vicuñas with domestic livestock in Allarpo could be facilitating helminth transmission in this region.

The high prevalence of *F. hepatica* is consistent with previous studies in the Andean region (Neyra et al., 2002; Samamé et al., 2016), where the presence of intermediate hosts and humid conditions favors its life cycle. In this regard, the higher prevalence of *F. hepatica* in Allarpo (79.2%) compared to Atuna (60.9%) could be related to environmental and management factors specific to each community. The presence of humid areas, such as wetlands and stagnant water bodies, promotes the proliferation of snails of the genus Lymnaea, essential intermediate hosts in the *F. hepatica* life cycle. Additionally, overgrazing in these areas can increase pasture contamination with infective metacercariae, raising the infection risk for vicuñas (Chacma, 2018). The *F. hepatica* infection in vicuñas has significant implications for their health and conservation. Liver damage associated with *Fasciola hepatica* infection can manifest as weight loss, anemia, and reduced body condition, affecting survival and productivity (Londoñe et al., 2009).

Juveniles showed higher infection frequencies than adults in both communities (90.9% versus 71.3%), a pattern commonly described in camelids and other ruminants (Valenzuela et al., 1998; Stevenson et al., 2022). Younger animals generally have less developed immune responses and often engage in more exploratory grazing, increasing their contact with contaminated vegetation. Although the differences observed in the present study were not statistically significant, the consistency of the trend suggests that juveniles represent a group requiring particular attention in monitoring and management plans. The greater susceptibility of juveniles to parasitic infections has also been attributed to their limited experience in selecting safe pastures and water sources. Thus, juveniles tend to consume vegetation in areas more exposed to fecal contamination, increasing their risk of helminth infection (Aguirre and Cafrune, 2007). The observed behavioral pattern, together with the consistently higher infection frequencies observed in juveniles, provides a basis for prioritizing targeted parasite control strategies in younger animals, which represent the highest-risk group within the management system (Silva-Díaz et al., 2015; Stevenson et al., 2022). The increased susceptibility observed in younger animals is consistent with broader evidence showing that helminths modulate and suppress key components of the host

immune response, making immunologically immature individuals more vulnerable to infection (Gazzinelli-Guimarães and Nutman, 2018).

Several aspects should be considered when interpreting these results. Age classification was based on field estimation rather than recorded birth data, which may affect the precision of age-related comparisons. In addition, the geometric mean (GM) values of 50.0 (50.0-50.0) reported for *Nematodirus* and *Trichuris* correspond to the minimum detection limit of the modified McMaster method (1 egg = 50 EPG). This does not indicate identical parasite burdens but rather infections at the lower threshold of detection. Unequal distributions across sex and age groups reflect natural chaccu capture patterns and can reduce statistical power in certain comparisons. Finally, the use of bivariate tests limits the ability to evaluate interactions among host, environmental, and management factors. More robust multivariate approaches would help clarify these relationships in future work.

The predominance of *F. hepatica* and the higher parasite load observed in Allarpo underscore the need for local, community-adapted control strategies. Efforts should focus on wetland areas and places with high snail density, as well as on coordinated parasite control for domestic livestock sharing the same grazing areas. Given that domestic livestock such as cattle and sheep share grazing grounds with vicuñas and act as reservoirs for multiple helminths, coordinated control actions between wildlife authorities and local herders are essential. Joint deworming schedules, improved livestock management near communal pastures, and proper handling of contaminated water sources may reduce cross-species transmission. Establishing clear community agreements that integrate livestock and wildlife health could enhance overall ecosystem resilience. Juveniles, given their consistently higher infection frequencies, should also receive priority in monitoring programs. Because fascioliasis may compromise body condition and productivity, potentially affecting fiber yield, a valuable resource for Andean communities (Londoñe et al., 2009), regular parasite surveillance should be incorporated into vicuña management and conservation efforts to ensure healthier populations and sustainable fiber production.

The close interaction between vicuñas and domestic livestock, especially sheep and cattle, may also contribute to parasite transmission, as interspecies contact can increase environmental contamination and facilitate shared infection cycles (Samamé et al., 2016). Community-based management strategies that include coordinated deworming of livestock, improved grazing rotation, and reduced access to wet areas during high-risk periods could help mitigate infection pressure in reintroduced vicuña populations

#### **CONCLUSION**

The frequency of gastrointestinal helminths in reintroduced vicuñas varied between the two communities studied, with a higher prevalence in Allarpo (82.0%) than in Atuna (69.2%). *F. hepatica* was the most prevalent parasite in both locations (60.9% in Atuna and 79.2% in Allarpo), followed by Strongyle-type eggs (STE), which were also more common in Allarpo (42.0%) than in Atuna (17.45%). Parasite load, expressed as the geometric mean (GM) of eggs per gram (EPG), was likewise significantly higher in Allarpo than in Atuna, reinforcing the notion that local environmental and management conditions strongly influence helminth transmission dynamics. Parasite identification in this study relied exclusively on coprological methods. Although these techniques provide dependable estimates of prevalence and parasite burden under field conditions, they do not allow species-level differentiation. Future studies incorporating larval culture, molecular diagnostics, or necropsy-based confirmation would improve taxonomic resolution and broaden our understanding of helminth diversity in reintroduced vicuña populations. Overall, the present findings emphasize the need to integrate continuous health surveillance and parasite control into vicuña reintroduction and conservation programs. Tailored management strategies—particularly in wetter areas that favor fascioliasis transmission and in zones where vicuñas coexist with domestic livestock—are essential to prevent the establishment of new infection reservoirs and to support the long-term sustainability of vicuña populations and fiber production in the Andean region.

#### **DECLARATIONS**

## Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

## Acknowledgments

The authors gratefully acknowledge the Regional Directorate of South American Camelids (DIRCAMS), Huancavelica, Peru, for the valuable technical and administrative support during the development of the present study.

## Authors' contributions

All authors approved the final version of the article before publication in the journal. Formal analysis, Erick Rojas, Alberto Najarro, Gumercindo Machuca and Sandra Bezada; investigation, Erick Rojas and Sandra Bezada; acquisition of financing, Erick Rojas, Gumercindo Machuca and Sandra Bezada; research, Erick Rojas, Alberto Najarro and Sandra Bezada; methodology, Erick Rojas and Sandra Bezada; project management, Erick Rojas; resources, Erick Rojas, Sandra Bezada, Gumercindo Machuca; supervision, Erick Rojas; drafting: original draft, Erick Rojas, Sandra Bezada and Gumercindo Machuca; Writing: proofreading and editing, Erick Rojas, Sandra Bezada and Gumercindo Machuca. All authors have read and agreed to the published version of the manuscript.

#### **Ethical considerations**

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by all the authors carefully. The authors confirm that no AI-generated content was used in the writing of the manuscript. The study design, data collection, analysis, and interpretation were entirely conducted by the authors.

## **Funding**

The present research did not receive external financial support. Field sampling was authorized and facilitated by DIRCAMS and supervised by SERFOR as part of the official chaccu activities. All laboratory analyses, transportation, and field expenses were covered directly by the authors

## **Competing interests**

The authors declare no conflict of interest.

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