

1 **Gastrointestinal parasites in working donkeys (*Equus asinus*) from Cusco, Peru: First**  
2 **report of *Fasciola hepatica* infection in equids from Southern Peru**

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24  
25 **Abstract**

26 **1) Introduction:** Working donkeys (*Equus asinus*) are rustic animals used in rural  
27 communities as a means of transport and cargo. They are raised under grazing conditions, with  
28 precarious health management, conditions that favor the presence of severe parasitosis. The  
29 present study aimed to identify gastrointestinal parasites infecting working donkeys from the  
30 Colcha-Paruro district, Cusco, Peru, in October 2022.

31 **2) Material and Methods:** A non-probabilistic convenience sampling method was employed,  
32 collecting fecal samples from 26 donkeys. The samples were taken to the Laboratory of  
33 parasitology, at the Universidad Nacional Agraria La Molina in isothermal boxes with ice  
34 packages. Once in the laboratory samples were analyzed using a modified flotation technique  
35 using salt-sugar solution, a modified McMaster method, and the Fluke Finder. Coprocultures  
36 and the Baermann technique were performed to obtain infective larvae (L3) for morphological  
37 identification of strongyles.

38 **3) Results:** General results showed a prevalence of 100% (26/26) for strongyles. Infective  
39 larvae identified 100% of cyathostomes. Moreover, 19.2% (5/26) were positive for *Fasciola*.  
40 *hepatica* and 7.7% (2/26) for *Parascaris* spp.

41 **4) Discussions:** This study constitutes the second report in Peru of natural infection with  
42 *Fasciola. hepatica* in donkeys, and the first one for the Southern region. This finding suggests  
43 its possible role in the epidemiology of fasciolosis in the area, including a role in the zoonotic  
44 infection. The high prevalence of cyathostomes reported confirms the importance of these  
45 strongyles in grass-fed equines and indicates deficiencies in health protocols. Future studies on  
46 possible anthelmintic resistance are recommended.

47 **5) Conclusion:** Our study underscores the need for more effective parasite control strategies  
48 to improve the health status of working donkeys. to reduce the impact of these infections on  
49 their welfare and to consider these animals in human fascioliasis control programs from the  
50 One-Health perspective.

51 **Keywords:** Helminths, strongyles, donkeys, liver fluke, fasciolosis, Peru

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## 54 1. Introduction

55 Donkeys (*Equus asinus*) are a very important livestock species in various regions of Peru, as  
56 they continue to be used as pack and working animals, despite technological advances and  
57 agrarian mechanization, mainly due to the difficulty in applying agricultural technologies in  
58 remote regions of the Peruvian territory. In this context, donkeys constitute a practical  
59 alternative. These animals are considered to be resilient and rustic; therefore, their sanitary  
60 management is empirical and deficient [1]. Donkeys, like horses and other equids, are  
61 susceptible to several parasitoses; many of them are common to the equine family. These  
62 parasites, in both their adult and larval stages, can affect the animals negatively [2].

63 Gastrointestinal parasites are a major group of parasites that affect equids, being the most  
64 frequent: roundworms (*Parascaris equorum*), large strongyles (*Strongylus* spp), small  
65 strongyles or cyathostomes (*Cyathostominae*), pinworms (*Oxiurus equi*), and *Strongyloides*.  
66 Strongyles (both large and small) are the most prevalent, pathogenic, and difficult to treat due  
67 to the rise of anthelmintic resistance [3]. The negative impact of strongyles is linked to their  
68 biological cycle, as part of it occurs in the gastrointestinal tract, which can generate severe  
69 lesions in the mucosa and blood vessels during larval migration. This can cause mild colic to  
70 the death of the animal in extreme cases [4].

71 Other major equine gastrointestinal parasites are *Eimeria*. spp., which cause equine coccidiosis,  
72 tapeworms (*Anoplocephala perfoliata* and *A. magna*), and trematodes (*Fasciola hepatica*) [5].  
73 For instance, *A. perfoliata* can cause clinical disease in equines, producing ulcers in the  
74 intestinal mucosa, spasmodic colic, and ileal impaction. [6]

75 Knowledge is scarce about the presence of gastrointestinal parasites in donkeys in Peru, with  
76 only one report for the northern region (Cajamarca) and no recent reports for the southern  
77 region. The characterization of the parasitic fauna present in these animals will enable the

78 design of more effective preventive health control and management strategies for a specific  
79 area, tailored to local conditions.

80 The objective of this study was to study the fauna and identify the main parasitic species in  
81 donkeys (*Equus asinus*) from the district of Colcha, province of Paruro, in the Department of  
82 Cusco.

83

## 84 2. Materials and methods

### 85 2.1. Location

86 This study was carried out during October 2022, in the Colcha district, Province of Paruro  
87 (71°50'52" West longitude and between 13°45'42" South latitude, Greenwich meridian)  
88 (Figure 1). The district covers high Andean areas and inter-Andean valleys. This district is  
89 located 64 kilometers Southeast of Cusco city, at 3068 meters above sea level.

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### 91 2.2. Animals

92 The animals sampled were Creole donkeys (*Equus asinus*), grass-fed, primarily used for  
93 transport and packing. The sample size was non-probabilistic for convenience, as the total  
94 donkey population in the area is currently unknown, and there were logistical difficulties in  
95 accessing all areas of the district. Due to these difficulties, the number of sampled animals was  
96 severely reduced. Inclusion criteria were: animals older than 3 months of age (average age at  
97 the start of grazing). Animals that had undergone internal deworming less than six months prior  
98 to sampling were excluded. A total of 26 animals were sampled, 19 of which were males and  
99 7 were females. Body condition, weight, sex, and age of each animal were registered during  
100 sampling

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103 **2.3. Sampling**

104 For sample collection, approximately 10 grams of feces were extracted directly from the  
105 rectum using plastic rectal palpation gloves and mineral oil as a lubricant. Each sample was  
106 immediately placed in polyethylene bags properly labeled. The bags were then immediately  
107 placed in an isothermal container with gel packs to keep the samples at refrigeration  
108 temperatures (4–6°C). Samples were taken to the Laboratory of Parasitology, Faculty of  
109 Animal Science, National Agrarian University of La Molina.



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124 **Figure 1 Cusco Department (Red) - Paruro Province - Colcha District (yellow dot**  
125 **pointed by black arrow). (Taken from Google Earth)**

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143 **Figure 2. Donkey (*Equus asinus*) rearing conditions in the Colcha district. Note the**  
144 **coexistence of these animals in the rural environment, where they live in streets (2a), and**  
145 **grazing areas (2b), highlighting their importance in daily life and local agricultural**  
146 **activities.**

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## 149 **2.5. Coprological tests**

### 150 **2.5.1. Modified flotation technique**

151 Samples were analyzed using a modified flotation test with a saturated salt and sugar solution  
152 to detect and identify parasitic egg and/or oocyst stages [7]. The flotation solution used in this

153 protocol is made of 400 g of commercial salt and 500 g of brown sugar, dissolved in one liter  
154 of distilled water (specific gravity=1.27). In brief, 2 to 5 grams of feces were homogenized in  
155 flotation solution using a mortar and pestle. This suspension was then filtered through four  
156 layers of gauze and poured into 15-ml centrifuge tubes, filling the tubes to obtain a positive  
157 meniscus. A coverslip was placed on the top of the tubes as a lid, and it stood for 10 minutes.  
158 Each coverslip was transferred to a slide and observed under a LEICA microscope (model  
159 DM500) at 4x and 10x magnifications.

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### 162 **2.5.2. Modified McMaster technique**

163 A modified McMaster technique was performed to count Strongyle type eggs (STE) per gram  
164 of feces (epg) [8]. In brief, 2 grams of feces were homogenized in 28 ml of salt and sugar  
165 saturated flotation solution and filtered through four layers of gauze and poured into a 60-ml  
166 plastic beaker. Using a 3-ml Pasteur pipette, the homogenized material was poured into a 2-  
167 chamber McMaster slide. The slide was observed under a LEICA microscope (model DM500)  
168 at 4x and 10x magnifications. The total number of eggs counted in both chambers was counted  
169 and multiplied by 50 to obtain the epg.

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### 171 **2.5.3. Modified FlukeFinder® Test**

172 This test was performed to detect *F. hepatica* eggs [9]. In brief, 2 g of feces was homogenized  
173 in 30 ml of tap water. This suspension was passed through the Flukefinder® apparatus by tilting  
174 the apparatus, tapping it gently, and adding tap water directly from the faucet. This procedure  
175 was repeated three times. The apparatus was subsequently disassembled and the interior side  
176 of the lower mesh was rinsed thoroughly, using a washing bottle, into a 15-ml centrifugation  
177 tube. The suspension was allowed to stand for 10 min. Sediment was placed in a Petri dish,

178 and a drop of methylene blue was added, and then observed under a stereoscope (Leica EZ4,  
179 Germany).

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#### 181 **2.5.4. Stool culture and Baermann technique**

182 To identify which type of strongyles (small or large) were present in the samples, a  
183 coproculture was performed, mixing the STE-positive feces with vermiculite to obtain  
184 infective larvae (L3) by means of the Baermann Technique.

185

#### 186 **2.5.5. Measurement of infective larvae**

187 Following the recovery of infective larvae (L3), a measurement process was performed using  
188 the Leica Application Suite (LAS) EZ software, adapted to a Leica DM500 microscope (Leica  
189 Microsystems). Infective larvae were identified by their morphological characteristics, as  
190 *Strongylus* spp. or cyathotomes [10].

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### 192 **2.6 Data analysis**

193 Data and variables (hpg, body condition, weight, sex, and age) of donkeys, and detected  
194 parasites were organized using Microsoft® Excel® for Microsoft 365 MSO (version 2412  
195 build 16.0.18324.20092) for analysis and formulation of statistical tables and prevalences.  
196 Python version 3.9 software was used for correlations. Scatter plots were created for epg's  
197 using the Graphpad Prism ( Graphpad Software, USA)

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## 200 **3. Results**

201 The results obtained indicate that 100% (26/26) of the sampled donkeys tested positive for the  
202 presence of strongyles (STE) (Figure 4a). A notable finding is the exclusive presence of

203 *Parascaris* spp. in females, while none were detected in any males. On the other hand, *F.*  
 204 *hepatica* (Figure 2b) was identified in both sexes (Table 1).

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207 **Table 1. Overall prevalence, and according to sex, of helminths found in *Equus asinus* (n=26) in**  
 208 **the District of Colcha, Cusco. October 2022**

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Parasite	Frequency		Prevalence	95% CI
	Females	Males		
<b>Strongyles (STE)</b>	7/7	19/19	100% (26/26)	86.8% - 100%
<i>Fasciola hepatica</i>	3/7	2/19	19.2% (5/26)	6.6% -39.4%
<i>Parascaris</i> spp.	2/7	0/19	7.7% (2/26)	0.9% - 25.1%

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212 Regarding the sex and age of the animals, all females (9/9) and males (17/17) were positive for  
 213 the presence of strongyles, a result that was repeated in adults and juveniles. It should be noted  
 214 that the predisposition to suffer high parasite loads is not directly linked to sex or age.

215 Furthermore, the egg count per gram of feces (epg) showed an overall average of 844.23 epg  
 216 (500-1000 epg). Regarding parasitic burden levels, determined by epg, according to Lason (as  
 217 cited in Mulwa *et al.*, 2020), this can be classified into low (<500 epg), medium (500-1000  
 218 epg), and high burden (>1000 epg) (Table 3). Despite the broad dispersion observed, some  
 219 individuals had high loads (>1500 epg), but the majority of the population is clustered between  
 220 500 and 1000 epg. Therefore, donkeys have a highly variable load, with some individuals  
 221 severely infected and others with lower levels (Figure 5). Identification of infective larvae (L3)  
 222 obtained by stool culture indicated that 100% of the observed L3s (Fig. 6) correspond to  
 223 cyathostomes (subfamily Cyathostominae).

224 Finally, associations between epg, weight and body condition were found, where the higher  
 225 the epg, the better the body condition with a positive compensation (0.32), while weight and  
 226 epg have a negative compensation (-0.22).

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229 **Table 2. Distribution of parasitic burdens, expressed as eggs per gram (epg) of strongyles (STE)**  
 230 **in *Equus asinus* (n=26) from the District of Colcha, Cusco. October 2022.**

Range	Positive/total	Proportion (%)
Low (<500 epg)	6/26	23.07%
Medium (500-1000 epg)	11/26	42.31%
High (>1000 epg)	26/9	34.62%

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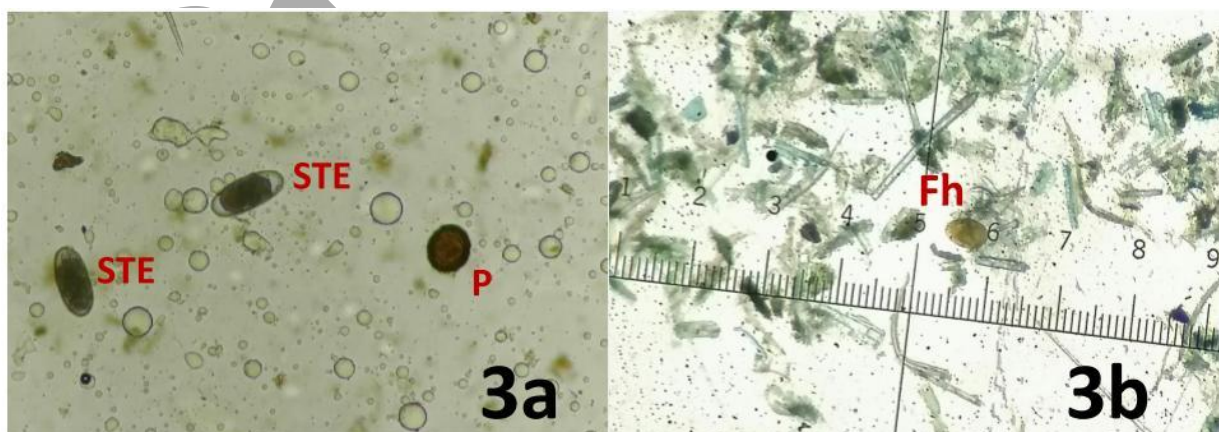
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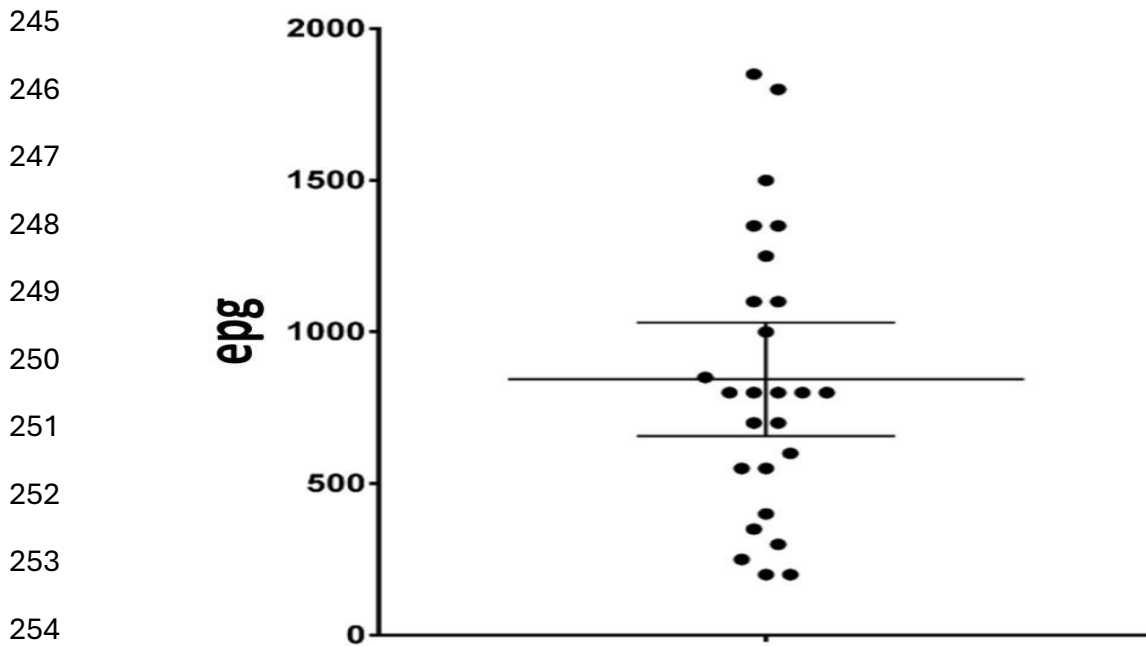
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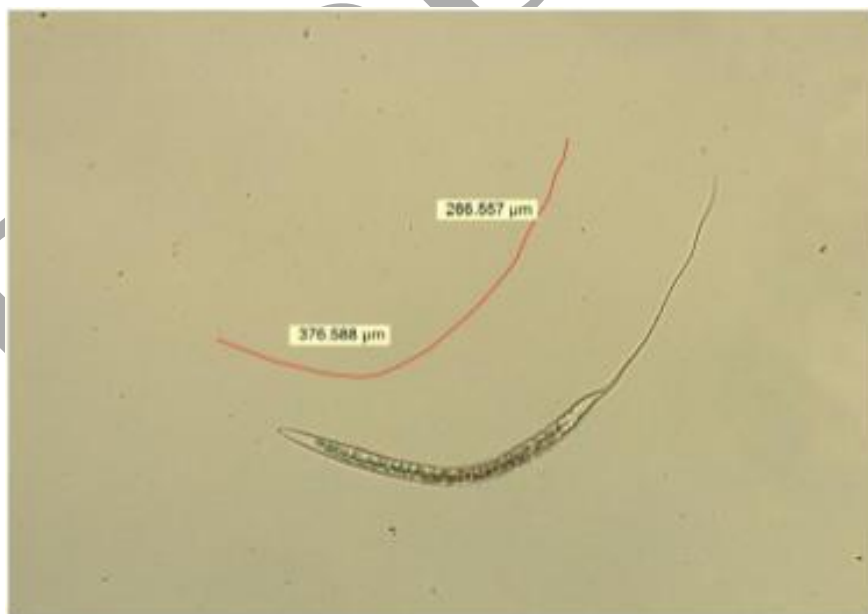
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242 **Figure 3. a ) Microscopic photograph (40X) of Strongyle type egg (STE) (STE) and *Parascaris***  
 243 **spp. egg (P) identified by the flotation test; ( b ) *Fasciola hepatica* egg (Fh) identified by the Fluke**  
 244 **Finder® test .**



255 **Figure 4 Dispersion of individual values of eggs per gram of feces (epg) of strongyles (STE)**  
 256 **obtained in *Equus asinus* (n=26) in the District of Colcha, Cusco, determined using the McMaster**  
 257 **technique in October 2022. Confidence interval 95%.**



270 **Figure 5: Infective larvae (L3) of cyathostomes (subfamily Cyathostominae) obtained using the**  
 271 **Baermann technique. Their respective measurements are in microns.**

#### 272 4. Discussions

273 Our results indicated a high overall prevalence for cyathostomes (100%), a moderate prevalence for the  
274 trematode *Fasciola hepatica* (19.2%), and a low percentage of infection with *Parascaris* spp. (7.7%).  
275 The prevalence of cyathostomes and *Parascaris* spp. observed in this study is similar to other previously  
276 reported in working donkeys in Latin America. For example, in Colombia Herrera *et al.* [11] reported  
277 a 98% prevalence for strongyles (large and small), and a 19.3% prevalence for *Parascaris equorum*. In  
278 Mexico, a 100% prevalence for gastrointestinal parasites, with a predominance of cyathostomes, has  
279 been reported [12]. On the other hand, in Ethiopia, strongyles are the most prevalent gastrointestinal  
280 parasite in working donkeys (37.74%); coincidentally with our results, *Parascaris* spp. was the second  
281 most common gastrointestinal helminth (11.28%) [13]. All these results confirm that cyathostomes  
282 (small strongyles) are the dominant parasites in donkeys worldwide. The similar high frequencies of  
283 cyathostomes reported in the Americas and Africa could be linked to the handling of donkeys, with  
284 non-optimal sanitary protocols, and the possibility of therapeutic failure of anthelmintic drugs [14].  
285 Regarding the burden of strongyles, expressed as fecal egg counts (epg), in Kenya, in a slaughterhouse  
286 for working donkeys it was found that 56% of slaughtered animals were not infected with intestinal  
287 helminths, 39% had a low burden (<500 epg), 5% a medium burden (501-1000 epg) and 0.7% a high  
288 burden (>1000 epg) [15]. In contrast, our results showed higher parasitic burdens, with 42.31% (11/26)  
289 animals exceeding 500 HPG and 34.62% (9/11) exhibiting epg's higher than 1000. These differences  
290 can be attributed to the fact that Kenya is one of the largest exporters of donkey meat in Africa. The  
291 use of donkey meat for human consumption implies more rigorous sanitary management. This is not  
292 the case in Peru, where the consumption of meat or derivatives of these animals is not part of the  
293 traditional diet and is even seen as taboo since these animals are perceived as working animals.  
294 The relatively low prevalence of *Parascaris* spp. found in our study could be related to the age of the  
295 animals since it has been demonstrated that this nematode usually occurs more frequently in populations  
296 of foals under one year old, as it is linked to the animal's poorly developed immunity and is not usually  
297 found in adults, as acquired immunity allows for better control [16]; in this way, the low frequency of

298 *Parascaris* spp. found by our study could be due to the small number of young animals (younger than  
299 one year) sampled during the study.

300 A remarkable finding of our study was the absence of common equine gastrointestinal helminths such  
301 as *Oxyuris equi*, *Trichostrongylus* spp. and *Anoplocephala* spp. These parasites have been documented  
302 in other countries in the Americas [11, 12]. Despite the greater variety of parasites reported in these  
303 investigations, cyathostomes were still the major group, followed by *Parascaris* spp. In our study, only  
304 three groups were found. This is possibly related to the reduced population of donkeys in the District  
305 of Colcha, over-dispersed and with very limited movement, therefore, with a low probability of  
306 contracting parasites. Another variable to be considered is specific local geographical and climatic  
307 conditions.

308 A notable discovery in this work is the detection of *Fasciola hepatica* in 19.2% (5/26) of the animals.  
309 This confirms the results obtained from previous studies conducted on donkeys and horses from  
310 Northern Peru [5, 17]. The donkey, like equines in general, lacks a gallbladder. This means that the bile  
311 produced by the liver is not stored and goes directly to the small intestine. Thus, from the point of view  
312 of the transmission of this trematode, the eggs cannot be stored in the gallbladder, and their fecal  
313 excretion should be more regular and constant than in animals that have a gallbladder [18]. The  
314 detection of fasciolosis in donkeys from this area highlights the risk of zoonotic transmission, as  
315 donkeys in the Andean rangelands interact with people and are in close contact with sheep and cattle,  
316 which are the primary reservoirs of this parasite. In this regard, the Colcha district reported a population  
317 of 2,086 cattle and 2,768 sheep [19], which shows the significant presence of potential hosts that may  
318 contribute to the presence of *F. hepatica* and their transmission to humans. The surveillance for human  
319 fascioliasis in Peru establishes guidelines for the detection and control of this zoonosis in vulnerable  
320 populations, highlighting equines as potential disseminators of the parasite in multiple regions [20]. It  
321 is important to mention that the donkey is the most susceptible equine species to suffer serious liver  
322 pathologies such as hyperlipidemia [18].

323 Finally, the correlations made between the epg's, body condition, and weight show that there is a weak  
324 but positive correlation between parasite burden and body condition (0.32), as well as a negative

325 relationship between epg and weight (-0.22). This differs from the common logic that would tell us that  
326 those animals with high parasitic burdens should present poor body condition and even some signs of  
327 clinical disease, however, this is explained by Morales *et al* [21] whose study carried out on grazing  
328 cattle shows similar results and explains that animals with good body condition are classified as  
329 "resilient", that is, they adapt to the high burdens finding a balance and maintaining their productivity,  
330 but it does not exempt them from being classified as disseminators of parasites and not being considered  
331 in deworming programs. On the other hand, the donkeys of the Peruvian highlands have dense, long  
332 fur, so we cannot rule out bias when visually judging body condition, which is subjective.

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334

### 335 **5. Conclusion**

336 Donkeys of the district of Colcha, Cusco have a high frequency of cyathostomes; in addition to  
337 *Parascaris* spp. and *Fasciola hepatica*, therefore, this species should be included in the epidemiological  
338 surveillance of trematode zoonosis. This study constitutes the second report of *Fasciola hepatica* for  
339 donkeys in Peru and the first one for the Southern region. Further future studies in more districts, with  
340 larger numbers of animals sampled, are recommended to better assess the gastrointestinal parasite fauna  
341 in donkeys from Southern Peru.

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343

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347

### 348 **Authors 'contribution:**

- 349
- MAVP: Data acquisition; data analysis; writing and editing
- 350
- IMLZ: Data acquisition; administrative support; statistical analysis; and supervision

351 • DAZR: Study concept and design; administrative support; data analysis; writing and  
352 editing; and supervision

353

354 **Conflict of interest:**

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

357

358 **Ethics approval:**

359 All procedures used for animal sampling were reviewed and approved by the Institutional  
360 Committee on Ethics in Animal Research and Biodiversity of the Universidad Científica del  
361 Sur (File No. 158-CIEI-AB-CIENTÍFICA-2021). All owners were informed about the research  
362 and the procedures that would be performed on their animals. To ensure clear and effective  
363 communication, a local translator was provided for illiterate and Quechua-speaking  
364 individuals, ensuring their understanding and informed consent.

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371

372 **Data availability:**

373 The data that support the findings of this study are available on request from the corresponding  
374 author, DAZR, due to privacy restrictions

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