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Original Article

Triclabendazole efficacy, prevalence, and re-infection of *Fasciola hepatica* in bovine and ovine naturally infected in the Andes of Ecuador

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ABSTRACT

Fasciola spp., infections are distributed worldwide including the Andes region of Ecuador, affecting cattle, sheep, porcine, humans, and other herbivores. Triclabendazole (TCBZ) is commonly used to treat animal infections. However, prospective studies on TCBZ efficacy and fascioliosis prevalence have not been studied in the highlands of Ecuador. This study was performed in a rural community at central of the Ecuadorian Andes in freely roaming bovine and ovine aimed to 1) evaluate the efficacy of TCBZ by administering a single oral dose of 12 mg/kg body weight, 2) assess the prevalence of *F. hepatica* infection and 3) to monitor re-infections for a follow-up period of five months. In total, 122, 86, 111, 110, 89, and 90 and 49, 34, 47, 28, 27, and 31 stool samples were collected each month from bovines and ovine, respectively. Besides, 32 stool samples from porcine were also collected at the beginning of the study. Stools were microscopically analyzed by formalin-ether concentration method to detect *F. hepatica* ova. The prevalence of *F. hepatica* infections before treatment was 55,7% and 63,3% for bovine and ovine, respectively. The infection prevalence was of 22% in porcine. The efficacity of triclabendazole was 83% and 97% in bovines and ovine, respectively, at 30 days post-treatment. The re-infection reaches to 54,4% in bovines and 61,3% in ovine after five months. TCBZ had a high efficacy and could be used for bovines and ovine *Fasciola* infections in the study region; however, re-infections reach the initial prevalence after five months. Therefore, we recommend integrated control strategies, including chemotherapy with a single oral dose of TCBZ, vector control, and future drug resistance studies.

1. Introduction

Triclabendazole (TCBZ), which was first introduced in the early 1980s, is the preferred anthelmintic for treating animal fasciolosis according to the World Organization for Animal Health (Fairweather, 2005). Several trials using a single oral dose of TCBZ (10–12 mg/kg) in naturally infected cattle and sheep have shown a significant reduction in fecal egg count, up to 90% after 28 days of administration in sheep and up to 95.4% in cattle 21 days post-treatment (García Chaviano et al., 2016; Kouadio et al., 2021; Tabari et al., 2022). However, treatment failure has increased in several studies on livestock, including cattle and sheep, and it has been reported in >11 countries (Carmona and Tort, 2017; Kelley et al., 2020; Ortiz et al., 2013). For human infections, TCBZ is the only medicine recommended by the World Health Organization (WHO, 2007). However, recent reports have shown that TCBZ has failed to cure fascioliasis in Peruvians (Morales et al., 2021). In Ecuador, TCBZ is formally registered for animal control by the Ministerio de Agricultura y Ganadería since 2017 to treat trematodes and nematodes in animals in 2017 (MAG, 2017); it is commonly used without veterinary prescription in pharmacies, even in small towns, either alone or in combination with other antiparasitic drugs, for treating livestock suspected to have fasciolosis.

Fasciolosis is a public health problem more common and widespread in animals than in humans (WHO, 2021). It is caused by two species, *Fasciola hepatica* and *F. gigantica* (WOAH, 2022), with only *F. hepatica* present in the Americas (WHO, 2021). Molecular

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characterization studies in Ecuador have also revealed that only *F. hepatica* is present in the country (Kasahara et al., 2021). Fascioliosis is endemic mainly in rural areas of the Andean region of Argentina, Bolivia, Peru, Ecuador, Venezuela, and Colombia, as well as in the highlands of Mexico (Mas-Coma et al., 2020). The most prevalent countries for livestock fascioliosis are the Andean regions of Peru, Bolivia, and Ecuador, with prevalence rates ranging from 15% to 66% (WHO, 2007).

Fasciola spp. is a trematode liver fluke distributed worldwide including the Andes region, affecting cattle, sheep, porcine, humans, and other herbivores (Carrada, 2007). The Andean region of Ecuador is considered endemic for fascioliosis in livestock above the 1800 m.a.s.l., with bovine and ovine prevalence rates varying from 0,59% to 60% (Enríquez-Ramos, 2020; Pavon, 2017; Velástegui and Guerra, 2012). Porcine are also reported to be infected (Kasahara et al., 2021; Mas-Coma et al., 2021; Rodríguez-Hidalgo et al., 2003). Human infections also occur in this region (Calvopiña et al., 2018; Trueba et al., 2000), where locals raise livestock using traditional management systems. Bovines, ovine, and porcine are fed in communal pastures with abundant natural freshwater sources and aquatic plants such as “berros” (*Nasturtium officinale*) (Celi-Erazo et al., 2020). In animals, the “gold standard” for diagnosing *Fasciola* infection is by observing the parasite, usually by examining *Fasciola* ova in stool specimens under a microscope.

In this study, we aimed to assess the efficacy of TCBZ in bovine and ovine after a 30-day treatment period and monitor the occurrence of any new infections during a 5-month follow-up period. Additionally, to investigate the prevalence of *Fasciola* infection in bovines, ovine, and porcine in a rural community in the Ecuadorian Andes.

2. Materials and methods

2.1. Study area

The study was conducted in a rural community situated in the central Andes of Ecuador (latitude: −2.260406 and longitude: −78.809767), at an altitude ranging between 2800 and 3520 m.a.s.l. The climate in this community is cold and temperate with temperature ranging from 5 °C to 17 °C. It presents precipitations throughout the year with an average between 450 and 1800 mm and a relative humidity of 71%. The location is described in detail and depicted on a map in (Celi-Erazo et al., 2020). According to the pre-study census, the animal population in the area comprised 175 bovines, 80 ovine, and 35 porcine, with agriculture and livestock being the main economic activities. Each family owned and managed a few heads of bovines, ovines, caprine, and porcine that grazed on permanent communitarian grasslands traditional maintained which rotate on demand in natural pastures over large areas

2.2. Study design

This investigation constitutes a longitudinal study spanning six months. The study protocol was formulated to gather samples at 30-day intervals or every 4 weeks. During the initial month, a fresh fecal sample was collected, and TCBZ treatment was administered regardless of the fascioliosis status of the population. In subsequent month, we assessed the prevalence of *Fasciola hepatica* and determined efficacy of the drug determining the number of individuals that were negative in comparison with the initial fecal examination. The subsequent period, encompassing the third to six months, was dedicated to follow-up observations and assessments of fascioliosis prevalence in the originally positive and negative animals. The community reported that no previous treatment has been administrated on their animals.

2.3. Fecal samples

In total, 122, 86, 111, 110, 89, and 90 and 49, 34, 47, 28, 27, and 31 fecal samples from bovines and ovine, respectively, were collected each month. Besides 32 fecal samples from porcine for one time at the beginning of the study. The prevalence of fascioliosis was determined by collecting approximately 10 g of fecal samples from the rectum of bovines, ovine, and porcine, using a sterile plastic bag. None of the animals examined in this study were inappropriate managed or experimented to obtained fecal samples. Samples were then analyzed microscopically using the formalin-ether sedimentation method to detect *Fasciola* ova (Ritchie, 1948). Briefly, 10 ml of distilled water was added to 2 g of feces. After homogenization, suspension was filtered with one layer of gauze and centrifuged at 1500 rpm for 2 min at room temperature. The sediment was then diluted with 5 ml of 10% formalin solution. After 10 min, 1 ml ether was added. The test tube was sealed, shaken vigorously for 60 s, and centrifuged again at 2000 rpm for 1 min at room temperature. After centrifugation, the supernatant was discharged. One drop of sediment was used for observation under a light microscope and observed with 5× and 10× lens.

2.4. Anthelmintic treatment and follow-up

We used a randomized open-label trial to assess the efficacy of TCBZ, based on two endpoints (Kaplan et al., 2023). First, at point-0 (first month), all bovines (122) and ovine (49) were weighed and administered a single oral dose of TCBZ (10% Over®)-Argentina, registered under Reg. San. 3A3–9944-SESA-U-Ecuador at a dosage of 12 mg/kg body weight by skilled veterinarians in two days. Thirty days after treatment (second month), stool samples were collected to determine the treatment efficacy by observing the presence or absence of *Fasciola* ova. Second, both positive and negative animals were screened for *Fasciola* ova prospectively for five consecutive months, with monthly stool examinations using the same formalin-ether fecal examinations. Porcine were excluded from TCBZ treatment and the prospective survey, as the prevalence of infection in this group is considered a high prevalence. Animals were treated in communal areas used for pasturage, without any other interventions such as changes in animal food habits. Treatment efficacy and follow-up infection at five months were determined based on the absence or presence of microscopic *Fasciola* ova, respectively. Animal management was carried out in coordination with the animal owners and all coprological testing and TCBZ treatment were provided free of charge.

2.5. Statistical analysis

The database was depurated in an excel sheet Microsoft Excel 2010. Prevalence was calculated as the numbers of positive individuals divided by the total population available at any point of the study (Jaramillo and Martínez, 2010; Thrusfield, 2018).

3. Results

In September, we detected *Fasciola* spp. ova in the three animal groups. In bovines, from 175 available, we examined 122 and detected ova in 68 (55.7%). For ovine, we examined 49 of the 80 available and detected ova in 31 (63.2%). Finally, from 35 porcine, we examined 32 and found 7 positive (21.9%; Fig. 1). Treatment was administered for positive and negative bovines and ovine (Table 1 and 2; Fig. 1). Porcine population remained untreated for the rest of the study.

For bovines, at the first-month follow up, 53 out of 68 positive animals were available for testing and only nine (17%) remained positive for *Fasciola* spp. ova detection (Table 1; Fig. 1). Among negative bovines, from 33 out of 54 examined, only one animal remained positive (3%; Table 1 and Fig. 1).

In the case of ovine, 22 positive individuals out of 31 were available for examination and only 2 (9.1%) remained positive. Conversely, from 18 negative, 12 were available for examination and only 4 (33.3%) were identified as positives (Table 2; Fig. 1).

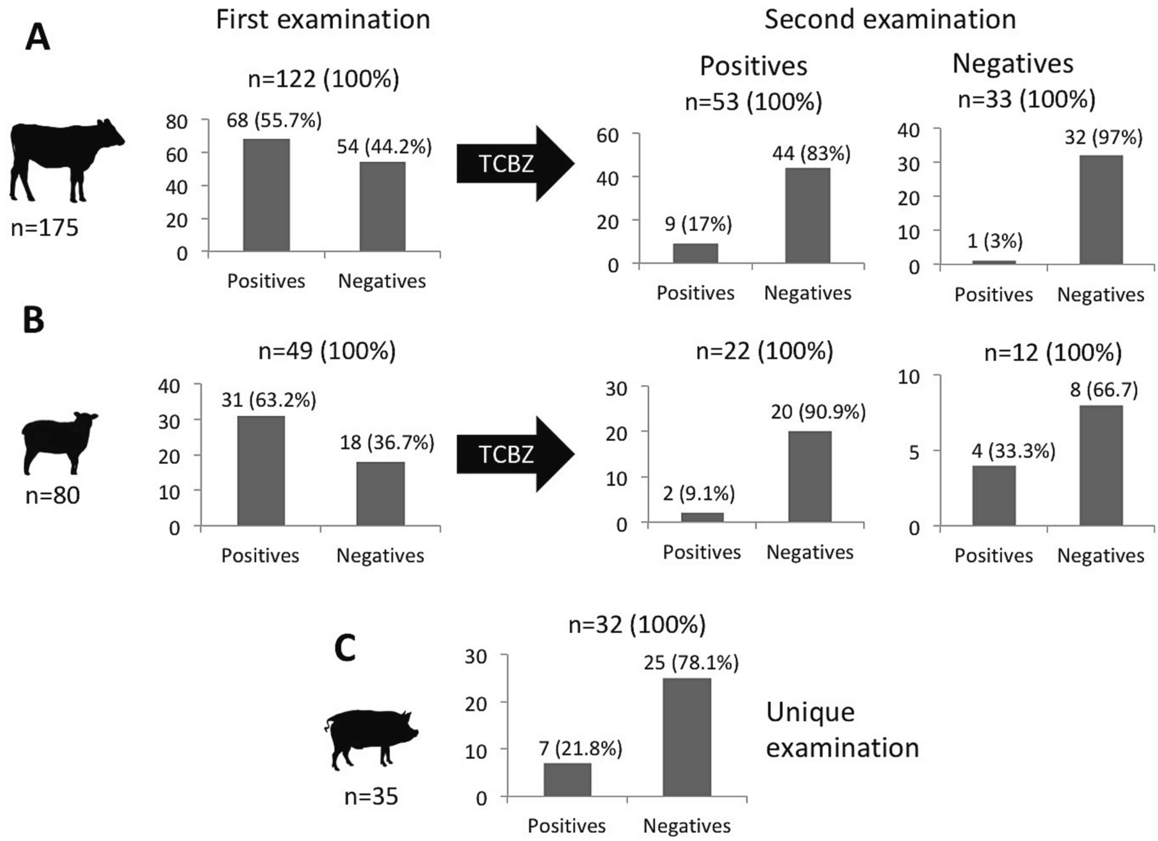

Fig. 1. Prevalence of *F. hepatica* infections and effectiveness of triclabendazole treatment. *Fasciola hepatica* prevalence was established after the examination of fecal samples of bovines, ovine, and porcine. Treatment success with TCBZ was evaluated 30 days after administration. (A) 122 bovines were examined for a prevalence of 55.7%; a subsequent examination demonstrated a prevalence of 17% among positive individuals and 3% among negative individuals. B) 49 ovine were examined for a prevalence of 63.2%; a subsequent examination demonstrated a prevalence of 9.1% among positive individuals and 33.3% among negative individuals. (C) 32 porcine were examined for a prevalence of 21.88%. Porcine were left untreated for fascioliosis.

Table 1
First examination and follow up of bovines examined in this study.

	Sept (pre-Tto)		Oct (post-Tto)	Nov	Dec	Jan	Feb
Positives	68 (55.74)	Positives	9 (16.98)	24 (38.71)	9 (14.29)	16 (31.37)	27 (50.94)
		Negatives	44 (83.02)	38 (61.29)	54 (85.71)	35 (68.63)	26 (49.06)
		Totals per category	53 (100)	62 (100)	63 (100)	51 (100)	53 (100)
Negatives	54 (44.26)	Positives	1 (3.03)	17 (34.69)	12 (25.53)	12 (35.58)	22 (59.46)
		Negatives	32 (96.97)	32 (65.31)	35 (74.47)	26 (68.42)	15 (40.54)
		Totals per category	33 (100)	49 (100)	47 (100)	38 (100)	37 (100)
Totals - first examination	122 (100)	Totals per month	86 (70.49)	111 (90.98)	110 (90.16)	89 (72.95)	90 (73.77)

September is the month of examination before the administration of triclabendazole (Tto; TCBZ). We administered TCBZ to the entire sampled population. Percentages are depicted in parenthesis. Percentages in the last row are calculated from the total of the first examination since the number of animals for follow up testing was variable.

Table 2
First examination and follow up of ovine examined in this study.

	Sept (pre-Tto)		Oct (post-Tto)	Nov	Dec	Jan	Feb
Positives	31 (63.27)	Positives	2 (9.09)	19 (65.52)	9 (45)	10 (50)	13 (61.90)
		Negatives	20 (90.91)	10 (34.48)	11 (55)	10 (50)	8 (38.10)
		Totals per category	22 (100)	29 (100)	20 (100)	20 (100)	21 (100)
Negatives	18 (36.73)	Positives	4 (33.33)	13 (72.22)	2 (25)	2 (28.57)	6 (60)
		Negatives	8 (66.67)	5 (27.78)	6 (75)	5 (71.43)	4 (40)
		Totals per category	12 (100)	18 (100)	8 (100)	7 (100)	10 (100)
Totals first examination	49 (100)	Totals per month	34 (69.39)	47 (95.92)	28 (57.14)	27 (55.10)	31 (63.27)

September is the month of examination before the administration of triclabendazole (Tto; TCBZ). We administered TCBZ to the entire sampled population. Percentages are depicted in since the number of animals for follow up testing was variable.

During the five-month follow up (October to February), availability for animal testing was variable ranging from 86 to 111 for bovines (>70%; Table 1) and from 28 to 47 for ovine (>55%; Table 2), thus, results are depicted in percentages (Fig. 2). For positive bovines detected after TCBZ treatment, prevalence increased from 16.98% (n = 9/53) in October to 50.94% (n = 27/53) in February (Fig. 2A; Table 1). Proportionally, those bovines identified as negatives after TCBZ treatment increased in positivity from 3.03% (n = 1/33) in October to 59.46% (n = 22/37) in February (Fig. 2B). Ovine presented a similar trend with positive individuals increasing in percentage from 9.09% (n = 2/22) in October to 61.9% (n = 13/21) in February (Fig. 2C); negative ovine after TCBZ treatment gradually increased in positivity from 33.3% (n = 4/12) in October to 60% (n = 6/10) in February (Fig. 2D; Table 2). Consistently, bovines and ovine detected as negatives after TCBZ administration (October) decreased in numbers by the end of the study period (Fig. 2; black lines). The owners reported no adverse events in any of the treatment animal groups.

4. Discussion

The efficacy of triclabendazole (TCBZ) was evaluated for the first time in an Andean community in Ecuador in a population of naturally infected bovines and ovine with fascioliosis. The high efficacy of TCBZ found 30 days post-treatment (83,02.8% and 96,97% in bovines and ovine, respectively) is consistent with other studies that reported efficacy ranging from 90% to 100% after 21 to 30 days post-treatment (García Chaviano et al., 2016; Kouadio et al., 2021; Tabari et al., 2022). However, it is worrisome that the follow-up by monthly incidence of *Fasciola* infection increased progressively, reaching up to 54,4% in and 61.3% in ovine after just 5 months of treatment, which is almost equal to the prevalence before treatment. There are several probable reasons for these reinfections and the initial high prevalence. Firstly, two intermediate host Lymnaea snail species, *Galba cousini* and *Galba schirazensis*, with high infection rates of *F. hepatica* are present in the surveyed area (Celi-Erazo et al., 2020). The latter is an invasive species with high transmission potential. Secondly, the study animals remained roaming free with access to contaminated aquatic plants and water, which could have contributed to reinfection. The extensive and community breeding system that predominates in the study area is favorable for *F. hepatica* infection, as animals are exposed to the infective *F. hepatica* metacercariae form. Moreover, in the highlands, the rainy periods are longer, which increases the presence of snails and the possibilities of infection.

Ovine exhibited the highest prevalence (63.27%; Table 2), potentially attributed to their prominent role as the primary livestock in the Ecuadorian highlands, owing to their robustness and adaptability compared to bovine animals (Quishpi Coronel, 2021). However, it is important to note that in this study, ovine there were less number of

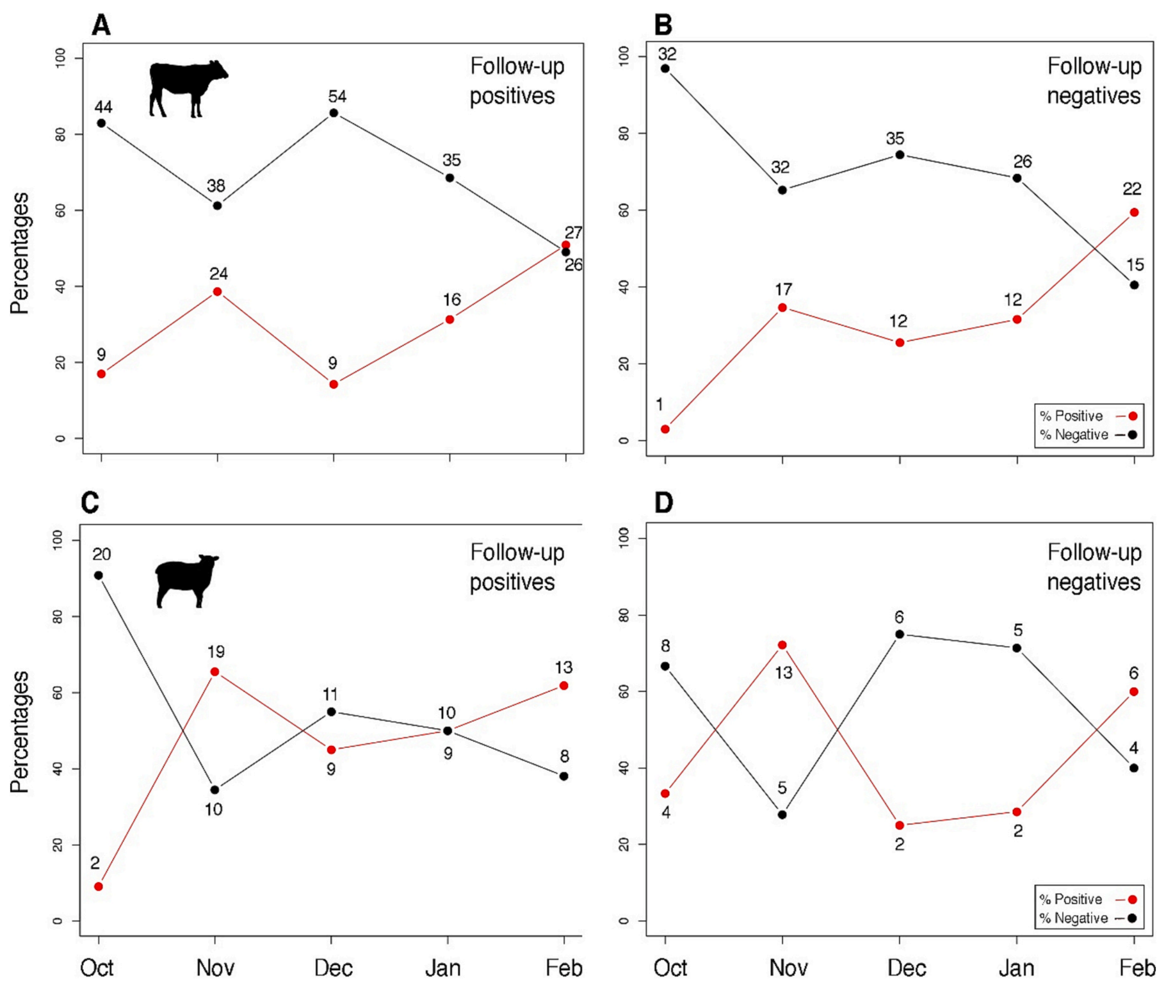

Fig. 2. Five-month follow-up of bovines and ovine treated with triclabendazole. After prevalence assessment, positive and negative animals were evaluated monthly for fascioliosis detection. For positive and negative bovines (A) and positive and negative ovine (B), prevalence progressively increased until the end of the study. For both populations negative individuals decrease progressively as well. Numbers in the plot represent the counts of animals included to calculate the percentage of positives (red lines) and negatives (black lines). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ovine were than bovine animals (i.e., 49 ovine vs. 122 bovines; Tables 1 and 2). Nonetheless, ovine are afforded greater freedom to graze and access aquatic plants. This is consistent with other studies conducted in Ecuador (Trueba et al., 2000) and Peru (Ortiz et al., 2013), which have also reported higher prevalence of fascioliosis in Andean ovine.

An important finding of our study was the high prevalence (22%) of *Fasciola* infection in porcine. Previous studies had not reported adult parasites in meat inspection or ova in coprological tests (Buestán, 2017; Gaona, 2015), suggesting that pig infection may be an under-recognized cause of liver disease in the Andean region. Porcine in the study area are free-roaming or tied with a rope in communal pastures where they come into contact with other ruminants, and they are also fed near fresh water sources where contaminated water and aquatic plants such as "berros" can infect them with *Fasciola* metacercariae. Only three slaughtered porcine in the northern Andes of Ecuador (Rodríguez-Hidalgo et al., 2003) and two specimens from the coastal region of Ecuador (Kasahara et al., 2021) had previously been reported with *Fasciola* infection.

5. Conclusion

Based on our results, the efficacy of TCBZ in a single dose had a high efficacy in bovines and ovine at 30 days post-treatment and could be used for their treatment. It was also evident that livestock fascioliosis is highly prevalent and endemic with active transmission in the central Andes region of Ecuador. Therefore, to control fascioliosis in the Andean region, we recommend implementing an integrated control program, which includes chemotherapy with a single oral dose of TCBZ for infected livestock, snail-vector control, education of the locals on improved animal husbandry, periodic monitoring of livestock, and raising awareness among veterinarians and health authorities.

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Ethical statement

After obtaining oral informed consent from the animal owners, fecal samples were collected by an experienced veterinarian without any inappropriate management or experimentation on the animals.

Declaration of Competing Interest

None.

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