SHORT COMMUNICATION

Efficacy of anthelmintic drugs to control *Fasciola hepatica* in dairy cattle in Peru

Eficacia de fármacos antihelmínticos para el control de Fasciola hepatica en ganado lechero en Perú

Eficácia de medicamentos anti-helmínticos no controle da Fasciola hepatica em bovinos leiteiros no Peru

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Abstract

Background: Decreasing antiparasitic efficacy of triclabendazole for controlling *Fasciola hepatica* in dairy cows in the Cajamarca Valley (Peru) has been reported. **Objective:** To determine the efficacy of four anthelmintic agents across a broader area of Cajamarca province. **Methods:** Four livestock farms were selected from three provinces in the Cajamarca region. Within each farm, 60 female cattle naturally infected with *F. hepatica* were chosen. Each farm was divided into four homogeneous groups based on individual animals and parasite burden. The groups were: triclabendazole (12 mg/kg of BW, VO), clorsulon/ivermectin (2 mg/kg and 0.2 mg/kg of BW, SC, respectively), closantel (10 mg/kg of BW, VO), and nitroxynil (10 mg/kg of BW, SC). Efficacy was determined following WAAVP guidelines by measuring the reduction in trematode egg shedding on day 30 post-dosing. **Results:** Triclabendazole demonstrated insufficient activity through FERCT and CPCR assessments across all four farms. The clorsulon/ivermectin and closantel groups exhibited high efficacy in all farms, while nitroxynil showed varying efficacy results in both types of analysis. **Conclusions:** Triclabendazole exhibited insufficient activity against *F. hepatica*. Clorsulon/ivermectin, closantel, and nitroxynil are viable alternatives with promising outcomes for controlling this trematode in the evaluated provinces.

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Keywords: *antiparasitic drugs; dairy cattle; bovine; Fasciola hepatica; fascioliasis; farm; parasitology, trematode.*

Resumen

Antecedentes: Se ha reportado una disminución de la eficacia antiparasitaria del triclabendazol en el control de *Fasciola hepatica* en vacas lecheras en el valle de Cajamarca, Perú. **Objetivo:** Determinar la eficacia antihelmíntica de cuatro antiparasitarios en un área más amplia de la provincia de Cajamarca. **Métodos:** Se seleccionaron cuatro predios ganaderos de tres provincias de la región Cajamarca. En cada predio se seleccionaron 60 hembras bovinas infectadas naturalmente por *F. hepatica*. Cada predio se dividió en cuatro grupos homogéneos según los individuos y la carga parasitaria. Los grupos fueron: triclabendazol (12 mg/kg de PV, VO), clorsulon/ivermectina (2 mg/kg y 0,2 mg/kg de PV, SC, respectivamente), closantel (10 mg/kg de PV, VO), y nitroxinil (10 mg/kg de PV, SC). La eficacia se determinó siguiendo las directrices de la WAAVP al medir la reducción en puesta de huevos de trematodos el día 30 posdosificación. **Resultados:** Por FERCT y CPCR, el triclabendazol fue insuficientemente activo en las cuatro explotaciones. Los grupos de clorsulon/ivermectina y closantel fueron altamente eficaces en todas las explotaciones y, finalmente, el nitroxinil mostró eficacia variada en ambos tipos de análisis. **Conclusiones:** El triclabendazol es insuficientemente activo frente a *F. hepatica*. Clorsulon/ivermectina, closantel, y nitroxinil presentan buenos resultados en el control de este trematodo en las provincias evaluadas.

Palabras clave: *bovino; fascioliasis; Fasciola hepatica; ganado lechero; medicamentos antiparasitarios; parasitología; trematodo.*

Resumo

Antecedentes: Foi relatada uma diminuição na eficácia antiparasitária do triclabendazol no controle da *Fasciola hepatica* em vacas leiteiras no Vale de Cajamarca, Peru. **Objetivo:** Determinar a eficácia anti-helmíntica de quatro antiparasitários em uma área maior da província de Cajamarca. **Métodos:** Foram selecionadas quatro propriedades pecuárias de três províncias da região de Cajamarca. Em cada propriedade, foram escolhidas 60 fêmeas bovinas naturalmente infectadas por *F. hepatica*; cada propriedade foi dividida em quatro grupos homogêneos com base nos animais individuais e na carga parasitária. Os grupos incluíram: triclabendazol (12 mg/kg kg de PV, VO), clorsulon/ivermectina (2 mg/kg e 0,2 mg/kg de PV, SC, respectivamente), closantel (10 mg/kg de PV, VO) e nitroxinil (10 mg/kg de PV, SC). A eficácia foi determinada seguindo as diretrizes da WAAVP ao medir a redução na excreção de ovos de trematódeos no dia 30 pós-dosagem. **Resultados:** Por meio das avaliações FERCT e CPCR, o triclabendazol demonstrou atividade insuficiente em todas as quatro propriedades. Os grupos de clorsulon/ivermectina e closantel exibiram alta eficácia em todas as propriedades, enquanto o nitroxinil apresentou resultados de eficácia variados em ambos os tipos de análise. **Conclusões:** O triclabendazol apresentou atividade insuficiente contra *F. hepatica*. Clorsulon/ivermectina, closantel e nitroxinil são alternativas viáveis com resultados promissores para o controle desse trematódeo nas províncias avaliadas.

Palavras-chave: *bovino; fasciolíase; Fasciola hepatica; gado leiteiro; medicamentos antiparasitários; parasitologia, trematódeo.*

Introduction

The trematode *Fasciola hepatica* has been reported in multiple countries worldwide to exhibit resistance to triclabendazole, which has long been the preferred drug for combating fascioliasis in both animals and humans (Cabada *et al.*, 2016; McMahon *et al.*, 2016; Ramadan *et al.*, 2019). Additionally, instances of resistance in specific regions have also been documented against albendazole (Sanabria *et al.*, 2013; Novobilský *et al.*, 2016; Ceballos *et al.*, 2019), as well as closantel (Novobilský and Höglund, 2015), and rafoxanide (Rapic *et al.*, 1988; Elitok *et al.*, 2006).

Association of metabolites with comparable activity and distinct mechanisms of action becomes imperative when pharmacological principles alone do not exhibit efficacy. This approach broadens the spectrum of activity of individual drugs, facilitating the treatment of mixed parasitosis or parasites belonging to the same phylum. Furthermore, such combinations can potentially delay the development of resistance to anthelmintics (Bartram *et al.*, 2012). Incorporation of active principles with differing mechanisms of action from diverse chemical groups enhances the likelihood of achieving synergistic effects (Geary *et al.*, 2012). Consequently, triclabendazole -the primary fasciolicide of choice- has been combined with various anthelmintics, including clorsulon, ivermectin, levamisole, luxabendazole, moxidectin, nitroxynil, oxfendazole, oxyclozanide, among others. Such combinations have demonstrated improved efficacy (Fairweather and Boray, 1999; Geurden *et al.*, 2012; Martínez-Valladares *et al.*, 2014; Khan *et al.*, 2017).

The World Association for the Advancement of Veterinary Parasitology (WAAVP) recommends methods and techniques for assessing antiparasitic efficacy. In controlled trials, efficacy is determined by comparing the number of live parasites in treated animals with that in untreated controls. However, in clinical trials involving live animals, efficacy is ascertained by comparing fecal egg count of treated animals to that of the same untreated animals shortly before or at the time of treatment and within a period not less than 3 weeks later (Wood *et al.*, 1995).

Similar to many regions worldwide, Cajamarca serves as a prominent cattle-raising area where dairy cattle breeds like Holstein Friesian, Brown Swiss, and Jersey are extensively reared. Nonetheless, numerous provinces within Cajamarca are marked by endemic fascioliasis, affecting both animals and humans (Cornejo *et al.*, 2010; Rodríguez-Ulloa *et al.*, 2018; Torrel *et al.*, 2023). This scenario has prompted prolonged anthelmintic usage, ultimately leading to the emergence of anthelmintic resistance due to persistent use of the same active ingredient. This situation is exemplified by triclabendazole, specifically within the Cajamarca district's valley, impacting dairy cattle (Ortiz *et al.*, 2013). However, this condition is unknown in other provinces, where evaluation of alternative fasciolicides has been unexplored. Thus, the present study assessed the efficacy of four chemical products, namely triclabendazole, nitroxynil, clorsulon/ivermectin, and closantel in four cattle farms within the Cajamarca provinces -Cajamarca, San Marcos, and San Miguel.

Materials and Methods

Ethical considerations

Farm owners were informed and gave written authorization for the use of their animals. In addition, all procedures were in accordance with the European ethical regulations for the use of animals in scientific research (European Directive 2010/63/EU).

Location

The study was conducted in four cattle farms located in three provinces of the Cajamarca region: Cajamarca (P-I and P-II), San Marcos (P-III), and San Miguel (P-IV) (Figure 1). Processing and diagnostic tests were performed at Laboratorio de Parasitología Veterinaria y Enfermedades Parasitarias, Facultad de Ciencias Veterinarias of Universidad Nacional de Cajamarca, Perú.

Experimental design

A cross-sectional study was conducted were initial sampling included all the animals within the farms, aiming to confirm positive cases and prevalence rates. From this, a cohort of 60 female cows, each exceeding eight months of age, was meticulously chosen. These cows were positive for presence of *F. hepatica* eggs in fecal matter, with a parasite load equal to or exceeding 1 egg per gram of feces (EPG). The selection process involved animals naturally infected within each farm; specifically, Jersey cows in the first farm and Holstein cows in the remaining three. Furthermore, the selected cows had not been subjected to anthelmintic administration during four months. They were maintained under similar conditions in terms of management and feeding, in an extensive breeding system.

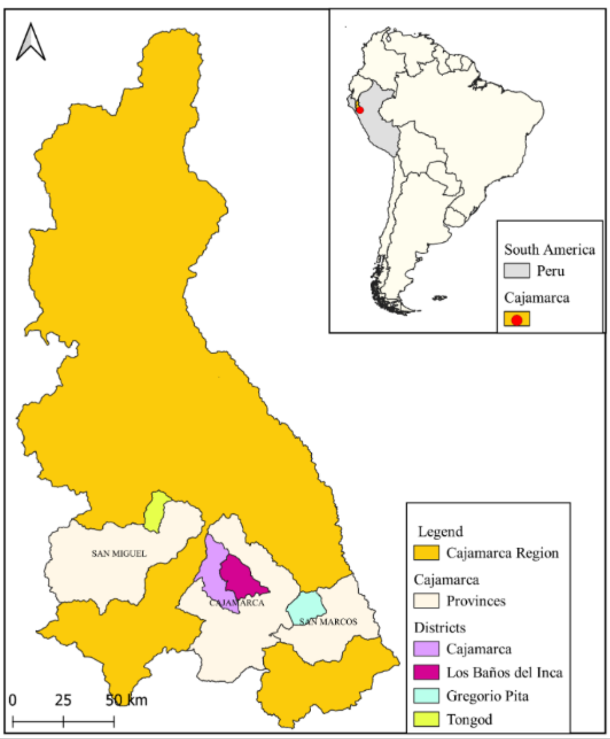

Figure 1. Location of the study.

Early in the morning (6:00 h) cows were weighted using bovine-specific metric tape tailored for the Jersey/Holstein breed. Simultaneously, fecal samples (approximately 100 g each) were directly retrieved from the rectum using veterinary obstetrical gloves. These samples underwent processing on the same day, employing the Rapid Sedimentation Technique as outlined by Lumbreras *et al.* (1962). Briefly, a homogenization procedure was employed by mixing four grams fecal matter with 40 mL running water within a conical-bottomed tube. This amalgamation was sieved into a 250 mL glass beaker, completing the volume with running water. The solution was then left to rest for 30 min. Subsequently, two-thirds of the supernatant was decanted and replenished with water for an identical resting period. This sequence was repeated until the supernatant exhibited apparent clarity. The ultimate sediment was augmented with two drops of methylene blue and placed within a Petri dish for examination using a stereomicroscope (3X, 4X increases).

Three days after obtaining the coproparasitological outcomes four groups were constituted per farm, each with the same number of individuals (n = 15). Each of these groups underwent administration of an antiparasitic agent. The composition of these groups was structured as follows: triclabendazole at a dose of 12 mg/kg of BW, VO (Bilevon® 12%, Bayer S.A. Lab. CIFARMA S.A., Peru); clorsulon at 2 mg/kg of BW, SC, and ivermectin at 0.2 mg/kg of BW, SC (Ivomec® F, Boehringer Ingelheim Animal Health do Brasil Ltda, Brazil); closantel at 10 mg/kg of BW, VO (Fasintel®10, Quimtia, Peru); and nitroxinil at 10 mg/kg of BW, SC (Nitromic 34%, Lab. Microsules Uruguay S.A., Uruguay). The volume administered was calculated by multiplying animal live weight by therapeutic dose of each active ingredient, and then dividing by the concentration of the product. Administration of clorsulon in conjunction with ivermectin did not intend to link these two substances; rather, it was due to absence of a commercially available product in the local market with solely the clorsulon active ingredient.

Sampling and analysis

At a third visit on day 30 post-dosing, fecal samples were extracted with the same procedure conducted during the first visit, and coproparasitological analyses were performed again by rapid sedimentation, and the Fecal Egg Count Reduction Test (FERCT) was applied.

Fecal samples were obtained using the identical procedure as employed during the initial sampling. Subsequently, coproparasitological analyses were reiterated utilizing the rapid sedimentation technique, resulting in the quantification of fecal egg count (FEC) which was expressed in eggs per gram (EPG).

Consequently, the anthelmintic effectiveness of each medication was assessed in accordance with the protocols stipulated by the WAAVP, employing the formulation for a fecal egg count reduction test (FECRT): %reduction = [($\bar{X}$ FEC day 0 - $\bar{X}$ FEC day 30) / $\bar{X}$ FEC day 0] x 100, where $\bar{X}$ represents the arithmetic mean. Categorization of product efficacy was defined as highly effective (>98%), effective (90-98%), moderately effective (80 - 89%), and insufficiently active (<80%) (Wood *et al.*, 1995). Simultaneously, the percentage of cattle positive by coprology reduction (CPCR) was also determined.

To establish more precisely the efficacy of flukicides in clinical trials involving *F. hepatica*, the WAAVP recommends calculating the FEC of treated animals over a period not less than 3 wk and comparing it to the FEC of the same animals prior to treatment or at the exact dosing time. This time frame is justified by the biological cycle of the trematode. In the early immature stage (1-4 wk), the trematode is migrating to the parenchyma, in the late immature stage (6-8 wk), it is in the prepatent period within the biliary duct, and in the mature stage (12-14 wk) it resides in the biliary ducts (Wood *et al.*, 1995). In other words, immature parasites do not mature and produce eggs within 30 d, which could introduce bias during the analysis.

The Wilcoxon test (for paired samples) was employed using the IBM SPSS Statistics 27.0.1 software to assess whether a statistical difference exists between EPG on day 0 and day 30. Differences or similarities in egg count between day 0 and day 30, as well as efficacies among the antiparasitic groups within each farm, were analyzed using the Kruskal-Wallis statistic. Following this test, the Mann-Whitney U test was employed to identify distinct groups within each farm in cases where the Kruskal-Walli's test detected statistical differences.

Results

Through FERCT and CPCR assessments, triclabendazole demonstrated insufficient activity across all farms. The clorsulon/ivermectin and closantel groups exhibited high efficacy in all farms, while nitroxynil showed varying efficacy results in both types of analysis (Table 1).

Discussion

According to the WAAVP, in clinical trials conducted on animals naturally infected with *F. hepatica* the control period must extend for at least 21 d (Wood *et al.*, 1995) because adult flukes could be dying and yet continue to release eggs, or the eggs stored in the bile duct could be eliminated. Upon reaching the 30-d mark -as in the present study- it is assured that adult trematodes have either died or become infertile as a result of the antiparasitic treatments. Furthermore, the possibility of a juvenile parasite developing into an adult and initiating egg laying within a 30-d timeframe is not likely. If any juvenile worm were to migrate to the bile ducts, it would perish due to the early blood consumption induced by the administered drugs. If it were to survive, it would take between two to three months to reach sexual maturity and commence egg production.

Although the WAAVP envisions studies on antiparasitic efficacy with control groups, it was not feasible in the present study since livestock farmers in the Cajamarca Valley follow a health calendar that entails three to four mandatory deworming treatments per year. They strictly adhere to this schedule and do not allow their animals to exceed these intervals, even though they do not perform diagnostic studies to confirm parasitic infections. If, for any reason, they detect that their animals are confirmed to have parasites, they become distressed and do not cooperate in establishing a control group, making it difficult to conduct comprehensive studies including control groups. Therefore, working with control groups is nearly impossible in this location unless the study involves the use of the researchers' own animals. Nonetheless, clinical studies comparing fecal egg counts between post-treatment and pre-treatment stages yield satisfactory results.

Table 1. Anthelmintic efficacy against *F. hepatica* within naturally infected cattle across three provinces within Cajamarca region.

Province	Prevalence (% ± 95%CI)	Drug	n	FERCT				CPCR	
				x̄ EPG ± 95%CI (day 0)	x̄ EPG ± 95%CI (day 30)	%Efficacy ± 95%CI	Condition	%Efficacy ± 95%CI	Condition
Cajamarca: P-I	80/117 (68.38 ± 8.43)	Triclabendazole	15	6.53 ± 3.62[ax]	2.47 ± 1.32[b]	62.24 ± 9.60[x]	IA	33.33 ± 23.86[x]	IA
		Clorsulon/Ivermectin	15	6.27 ± 3.03[ax]	0.00 ± 0.00[b]	100 ± 0.00[y]	HE	100 ± 0.00[y]	HE
		Closantel	15	5.93 ± 3.74[ax]	0.00 ± 0.00[b]	100 ± 0.00[y]	HE	100 ± 0.00[y]	HE
		Nitroxynil	15	7.13 ± 2.82[ax]	0.07 ± 0.13[b]	99.07 ± 1.82[z]	HE	93.33 ± 12.63[z]	E
Cajamarca: P-II	62/76 (81.58 ± 8.72)	Triclabendazole	15	7.93 ± 5.78[ax]	6.47 ± 5.65[b]	18.49 ± 6.98[x]	IA	20 ± 20.24[x]	IA
		Clorsulon/Ivermectin	15	7.4 ± 3.17[ax]	0.00 ± 0.00[b]	100 ± 0.00[y]	HE	100 ± 0.00[y]	HE
		Closantel	15	7.8 ± 3.99ax	0.00 ± 0.00[b]	100 ± 0.00[y]	HE	100 ± 0.00[y]	HE
		Nitroxynil	15	9.13 ± 3.73ax	0.06 ± 0.13[b]	99.27 ± 1.43[z]	HE	93.33 ± 12.63[z]	E
San Marcos: P-III	65/95 (68.42 ± 9.35)	Triclabendazole	15	6.13 ± 3.44ax	3.07 ± 2.50[b]	50.00 ± 10.22[x]	IA	26.67 ± 22.38[x]	IA
		Clorsulon/Ivermectin	15	8.13 ± 3.49[ax]	0.00 ± 0.00[b]	100 ± 0.00[y]	HE	100 ± 0.00[y]	HE
		Closantel	15	7.07 ± 3.63[ax]	0.00 ± 0.00[b]	100 ± 0.00[y]	HE	100 ± 0.00[y]	HE
		Nitroxynil	15	5.73 ± 2.92[ax]	0.4 ± 0.32[b]	93.02 ± 5.39[z]	E	66.67 ± 23.86[x]	IA
San Miguel: P-IV	69/124 (55.65 ± 8.74)	Triclabendazole	15	18.47 ± 5.01ax	16 ± 5.43[a]	13.36 ± 4.30[x]	IA	0.00 ± 0.00[x]	IA
		Clorsulon/Ivermectin	15	20.07 ± 5.71[ax]	0.00 ± 0.00[b]	100 ± 0.00[y]	HE	100 ± 0.00[y]	HE
		Closantel	15	19.13 ± 7.81[ax]	0.00 ± 0.00[b]	100 ± 0.00[y]	HE	100 ± 0.00[y]	HE
		Nitroxynil	15	18.07 ± 8.08[ax]	0.00 ± 0.00[b]	100 ± 0.00[y]	HE	100 ±0.00[y]	HE

*EPG: Eggs per gram o feces

[a,b]Distinct letters within the same row indicate statistical differences in EPG counts between day 0 and day 30 (Wilcoxon test, $p<0.05$).

[x,y,z]Distinct letters within the same column within each farm indicate statistical differences in efficacies (Kruskal-Wallis + Mann-Whitney U post hoc, $p<0.05$).

Dosage: Triclabendazole 12 mg/kg, clorsulon 2 mg/kg, closantel 10 mg/kg, nitroxynil 10 mg/kg, ivermectin 0.2 mg/kg.

Categorization: Insufficiently active (IA), effective (E), and highly effective (HE).

The prevalence of *F. hepatica* in the farms ranged from 55.65 ± 8.74% in San Miguel to 81.58 ± 8.72% in Cajamarca. These results are not novel, as Cajamarca, particularly the Cajamarca Valley, is recognized as an endemic area for fasciolosis due to favorable environmental conditions for the intermediate host and extensive breeding. Records of trematodes in animals exist, even predating 1998 (Claxton *et al.*, 1998). Various districts in Cajamarca have reported diverse prevalences of *F. hepatica* in cattle. In more distant districts from the Cajamarca Valley, such as Chota, a prevalence of 20.3 ± 4% has been found, 45.5 ± 5% in Celendín, 50 ± 5% in San Juan, and in close areas, 80.7 ± 4.1% in La Encañada, 61.2 ± 5.6% in Los Baños del Inca, and 49.5 ± 5% in hamlets of the Cajamarca district (Torrel *et al.*, 2023).

Due to high prevalence of this trematode in the Cajamarca Valley, livestock farmers have the habit of deworming their animals against *F. hepatica* every three or four months. Due to the fact that triclabendazole acts on various stages of the trematode (Cwiklinski and Dalton, 2018), it has been indiscriminately used for a long time without technical considerations throughout the local livestock sector. Reports of its use even precede 1998 (Claxton *et al.*, 1998). The average livestock owner does not conduct clinical efficacy tests of the antiparasitics used. This responsibility falls on non-professional livestock personnel who often fail to accurately calculate therapeutic doses and neglect drug rotation. This situation is consistent with observations by other researchers who have mentioned that resistance tends to develop when the parasite control relies exclusively on the same active ingredient over an extended period, with high frequency of deworming, suboptimal dosing, indiscriminate use of antiparasitics, lack of drug rotation, and the absence of comprehensive technical criteria (Márquez, 2003; Anziani and Fiel, 2015).

Due to these circumstances, triclabendazole was reported to be inadequately effective in controlling *F. hepatica* in dairy cattle within the Cajamarca Valley as early as 2012. In one farm (Tartar) 2.8% efficacy was observed; 3.1% in the second (El Cortijo); and 68% in a third (San Vicente) (Rojas-Moncada, 2012). A year later in the same Valley, in a more rigorous study, triclabendazole achieved 31.05% efficacy on day 14 and 13.63% on day 30 in cattle (Ortiz *et al.*, 2013). Simultaneously, reports of *F. hepatica* resistance to triclabendazole surfaced in other regions of Peru (Chávez *et al.*, 2012). This phenomenon has also been observed worldwide (Olaechea *et al.*, 2011; Brockwell *et al.*, 2013; Coyne *et al.*, 2020; Kelley *et al.*, 2020). Even at a concentration of 24 mg/kg, double the usual dose, satisfactory results have not been achieved (Romero *et al.*, 2019). Nevertheless, in areas where its use is not widespread or where its introduction is recent, triclabendazole maintains optimal efficacy (Kouadio *et al.*, 2021).

The local livestock owners with better resources engage in improved livestock management and receive guidance from veterinarians to implement strategic antiparasitic administration, including drug rotation, which might explain the high efficacy of clorsulon/ivermectin, closantel, and nitroxinil. Furthermore, these drugs are relatively new in the local market compared to triclabendazole. Other researchers have also found satisfactory efficacy results. Clorsulon has been used as an alternative to eliminate the adult phase of parasites resistant to triclabendazole (Elliott *et al.*, 2015). While closantel has shown excellent results in the present study and in other regions (Borgsteede *et al.*, 2008; Nzalawahe *et al.*, 2018; Bushra *et al.*, 2019), therapeutic failures have also been reported (Novobilský and Höglund, 2015). On the other hand, nitroxinil has yielded optimal results in the therapeutic management of bovine fascioliasis and represents an alternative in cases of triclabendazole resistance in cattle (Wood *et al.*, 1995; Martínez-Valladares *et al.*, 2010).

Several researchers report that antiparasitic products with efficacy below 90-95% still hold value, even if not 100% effective, as they substantially reduce parasite burden or reach an economic threshold, thus not significantly impacting animal health and productivity (Fairweather, 2011; Forbes, 2013). However, in the present study triclabendazole did not reach even 20% efficacy; hence, its use should be ceased to avoid unnecessary losses, given that the cost of bovine fasciolosis infection can be quite high, manifesting as decreased fertility, reduced weight gain, diminished milk production, liver condemnations, and poor carcass performance (Schweizer *et al.*, 2005; Sariözkan and Yalçın, 2011; Charlier *et al.*, 2012; Fanke *et al.*, 2017). Therefore, control schemes must be cost-effective, and drug administration should be judicious, accompanied by regular clinical efficacy studies. Nevertheless, controlling *F. hepatica* requires an integrated approach considering the epidemiological triad. For instance, cattle are less infected with *F. hepatica* in sprinkler-irrigated pastures, in contrast to flood-irrigated pastures, which is a common practice in Cajamarca (Torrel-Pajares *et al.*, 2023).

Although emphasis is being placed on current techniques for evaluating *F. hepatica* resistance and several diagnostic methods are available, recommended guidelines and standardized protocols are lacking (Fairweather *et al.*, 2020). Molecular techniques can be employed to identify molecular markers of resistance, along with simpler methods such as the controlled efficacy test (CET), fecal egg count/reduction test (FEC/FECRT), coproantigen reduction test (CRT), and egg hatching assay (EHA). The CRT has proven to be a solid alternative to FECRT for evaluating triclabendazole resistance of *F. hepatica* in cattle, and its use involves employing an ELISA kit (Brockwell *et al.*, 2013). However, the CRT and other techniques entail higher costs compared to FECRT, which can be implemented in basic laboratories and field settings, accessible to most professionals with non-sophisticated and cost-effective technology. Nonetheless, further studies comparing these techniques are necessary to define the best method for assessing drug resistance of *F. hepatica*.

In conclusion, triclabendazole is insufficiently effective against *F. hepatica*. Antiparasitics based on clorsulon/ivermectin, closantel or nitroxinil show good results for controlling the trematode in the studied zones of Cajamarca region. However, they should be carefully used -including rotation and regular clinical evaluations- to prevent antiparasitic resistance.

Declarations

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Conflict of interest

The authors declare that they have no known financial interests or personal relationships that could have influenced the work presented in this article.

Author contributions

JRM and ST conceptualized, designed the methodology, supervised and managed the research. LS, VU, RV, and AR Executed and carried out field and laboratory work. LV-R and CM-M contributed to the software, validation, data curation and writing-preparation of the original drafts. All authors collaborated in the visualization, writing-revising and editing of the manuscript. All authors approved the final manuscript and accepted responsibility for its content.

Use of artificial intelligence (AI)

No AI or AI-assisted technologies were used during the preparation of this work.

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