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*

Juan Rojas-Moncada¹; Luz Saldaña¹; Víctor Urteaga¹; Roxana Vergara¹; Anthony Rojas¹; Severino Torrel¹; César Murga-Moreno²,³; Luis Vargas-Rocha¹,³*

¹Laboratorio de Parasitología Veterinaria y Enfermedades Parasitarias, Facultad de Ciencias Veterinarias, Universidad Nacional de Cajamarca, Av. Atahualpa 1050, 06003 Cajamarca, Perú.

²Laboratorio de Inmunología e Investigación en Ciencias Veterinarias, Facultad de Ciencias Veterinarias, Universidad Nacional de Cajamarca, Av. Atahualpa 1050, 06003 Cajamarca, Perú

³Círculo de Estudios e Investigación en Ciencias Veterinarias - CEICIVET, Facultad de Ciencias Veterinarias, Universidad Nacional de Cajamarca, Av. Atahualpa 1050, 06003 Cajamarca, Perú.

* Corresponding author: Av. Atahualpa N° 1050. Facultad de Ciencias Veterinarias - Campus UNC. CEP 060003. Cajamarca, Peru: E-mail: lvargasr17_1@unc.edu.pe

Received: November 23, 2022. Accepted: September 8, 2023

This work is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License, which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

© 2023 Universidad de Antioquia. Published by Universidad de Antioquia, Colombia.
eISSN: 2256-2958
**Abstract**

**Background:** Decreasing antiparasitic efficacy of triclabendazole in 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 included: 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:** Through FERCT and CPCR assessments, triclabendazole demonstrated insufficient activity 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.

**Keywords:** antiparasitic drugs; dairy cattle; bovine; control; effectiveness; *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 nitroxynil (10 mg/kg de PV, SC). La eficacia se determinó siguiendo las directrices de la WAAVP al medir la reducción en...
la puesta de huevos de trematódos en 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 eficacias variadas en ambos tipos de análisis. **Conclusiones:** El triclabendazol es insuficientemente activo frente a *F. hepatica*. Clorsulon/ivermectina, closantel y nitroxinil son alternativas con buenos resultados en el control de este trematódeo en las provincias evaluadas.

**Palabras clave:** bovino; control; eficacia; fascioliasis; *Fasciola hepatica*; fundo; ganado lechero; medicamentos antiparasitarios; parasitología; trematódeo.

**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-helmintica 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; controle; eficácia; fasciolíase; fazenda; *Fasciola hepatica*; gado leiteiro; medicamentos antiparasitários; parasitologia, trematódeo.

**Introduction**

The trematode *Fasciola hepatica* has been reported to exhibit resistance to triclabendazole in multiple countries worldwide, 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 have also been documented against albendazole in specific regions (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).

When pharmacological principles alone do not exhibit efficacy, the association of metabolites with comparable activity and distinct mechanisms of action becomes imperative. 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 the involved anthelmintics (Bartram et al., 2012). The 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, the primary fasciolicide of choice, triclabendazole, has been combined with various anthelmintics, including clorsulon, ivermectin, levamisole, luxabendazole, moxidectin, nitroxynil, oxendazole, oxyclozanide, among others. Such combinations have demonstrated improved efficacy outcomes (Fairweather and Boray, 1999; Geurden et al., 2012; Martínez-Valladares et al., 2014; Khan et al., 2017).

The recommended methods and techniques for assessing the efficacy of an antiparasitic are provided by the guidelines of the World Association for the Advancement of Veterinary Parasitology (WAAVP). 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 the 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 w 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 reliance on 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, investigations into this scenario within other provinces have remained lacking, with the evaluation of alternative fasciolicides yet unexplored. Thus, the present study undertakes the assessment of the efficacy of four chemical products, namely triclabendazole, nitroxynil, clorsulon/ivermectin, and closantel. This evaluation is conducted across four cattle farms within the Cajamarca region's three provinces-Cajamarca, San Marcos, and San Miguel.
Materials and methods

Ethical considerations

The 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 province (P-I and P-II), San Marcos province (P-III), and San Miguel province (P-IV) (Figure 1). The processing and diagnostic tests were performed at the Laboratorio de Parasitología Veterinaria y Enfermedades Parasitarias, Facultad de Ciencias Veterinarias from the Universidad Nacional de Cajamarca.

Figure 1. Location of the study site.

Experimental design
This constitutes a cross-sectional investigation. The initial sampling encompassed all animals within the farms, aiming to confirm the positive cases and their respective prevalence rates. From this, a cohort of 60 female cows, each exceeding 8 months in age, was meticulously chosen. These cows exhibited a positive status for the presence of Fasciola 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 found to be 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 for a duration of four months. They were also maintained under similar conditions in terms of management and feeding, in an extensive breeding context.

At the early hours of the morning (6 a.m.), the individual weights of the cows were ascertained utilizing bovine-specific metric tape tailored for the Jersey/Holstein breed. Simultaneously, fecal samples, comprising approximately 100 g each, were directly retrieved from the rectum using veterinary obstetrical gloves. These collected samples underwent processing on the same day, employing the Rapid Sedimentation Technique as outlined by Lumbreras et al. (1962). In summary, a homogenization procedure was employed, involving the mixing of 4 g of fecal matter with 40 mL of running water within a conical-bottomed tube. This amalgamation was sieved into a 250 mL glass beaker, with the volume completed using running water. The solution was then left to rest for a duration of 30 min. Subsequently, two-thirds of the supernatant was decanted and replenished with water for an identical resting period. This sequence of steps was reiterated 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).

During a subsequent visit, 3 d after the acquisition of coproparasitological outcomes, four distinct groups were constituted for each farm, each comprising an equivalent 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 to be administered was computed by multiplying the animal's live weight by the therapeutic dose of each active ingredient, followed by division by the concentration of the product. The selection to administer clorsulon in conjunction with ivermectin was not driven by an intent to link these two substances. Rather, it was adopted due to the absence of a commercially available product featuring solely the clorsulon active ingredient within the local market.
Sampling and analysis

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

On the thirtieth day following dosing, a third visit was conducted. 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): 
\[
\text{% reduction} = \left( \frac{X_{\text{FEC day 0}} - X_{\text{FEC day 30}}}{X_{\text{FEC day 0}}} \right) \times 100
\]
where $X$ represents the arithmetic mean. The categorization of product efficacy was ascertained to be highly effective (>98%), effective (90-98%), moderately effective (80 - 89%), and insufficiently active (<80%) (Wood et al., 1995). Concurrently, the percentage of cattle positive by coprology reduction (CPCR) was also determined.

To establish the efficacy of flukicides more precisely in clinical trials involving *F. hepatica*, the WAAVP recommends calculating the FEC of treated animals over a period not less than 3 w 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 w), the trematode is migrating to the parenchyma, in the late immature stage (6-8 w), it is in the prepatent period within the biliary duct, and in the mature stage (12-14 w), it resides in the biliary ducts (Wood et al., 1995). In other words, an immature parasite does not mature and produce eggs within 30 d, which could introduce bias during the analysis.

To assess whether a statistical difference exists between the EPG on day 0 and day 30, the Wilcoxon test (for paired samples) was employed using the IBM SPSS Statistics 27.0.1 software. The differences or similarities in egg count between day 0 and day 30, as well as the 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-Wallis’s test detected statistical differences.

Results
Through FERCT and CPCR assessments, triclabendazole demonstrated insufficient activity 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 (Table 1).
Table 1. Anthelmintics efficacy in managing *F. hepatica* within naturally infected cattle across three provinces within Cajamarca region.

<table>
<thead>
<tr>
<th>Province</th>
<th>Prevalence (%)</th>
<th>Drug</th>
<th>n</th>
<th>$%$ EPG ± CI95% (day 0)</th>
<th>$%$ EPG ± CI95% (day 30)</th>
<th>Efficacy (%)</th>
<th>Condition</th>
<th>Efficacy ± CI95%</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cajamarca: P-I</td>
<td>80/117 (68.38 ± 8.43)</td>
<td>Triclabendazole</td>
<td>15</td>
<td>6.53 ± 3.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.47 ± 1.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62.24 ± 9.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>IA</td>
<td>33.33 ± 23.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>IA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Clorsulon/Ivermectin</td>
<td>15</td>
<td>6.27 ± 3.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100 ± 0.00&lt;sup&gt;y&lt;/sup&gt;</td>
<td>HE</td>
<td>100 ± 0.00&lt;sup&gt;y&lt;/sup&gt;</td>
<td>HE</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Closantel</td>
<td>15</td>
<td>5.93 ± 3.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100 ± 0.00&lt;sup&gt;y&lt;/sup&gt;</td>
<td>HE</td>
<td>100 ± 0.00&lt;sup&gt;y&lt;/sup&gt;</td>
<td>HE</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nitroxynil</td>
<td>15</td>
<td>7.13 ± 2.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.07 ± 0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>99.07 ± 1.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>HE</td>
<td>93.33 ± 12.63&lt;sup&gt;z&lt;/sup&gt;</td>
<td>E</td>
</tr>
<tr>
<td>Cajamarca: P-II</td>
<td>62/76 (81.58 ± 8.72)</td>
<td>Triclabendazole</td>
<td>15</td>
<td>7.93 ± 5.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.47 ± 5.65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.49 ± 6.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>IA</td>
<td>20 ± 20.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>IA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Clorsulon/Ivermectin</td>
<td>15</td>
<td>7.4 ± 3.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100 ± 0.00&lt;sup&gt;y&lt;/sup&gt;</td>
<td>HE</td>
<td>100 ± 0.00&lt;sup&gt;y&lt;/sup&gt;</td>
<td>HE</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Closantel</td>
<td>15</td>
<td>7.8 ± 3.99&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100 ± 0.00&lt;sup&gt;y&lt;/sup&gt;</td>
<td>HE</td>
<td>100 ± 0.00&lt;sup&gt;y&lt;/sup&gt;</td>
<td>HE</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nitroxynil</td>
<td>15</td>
<td>9.13 ± 3.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.06 ± 0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>99.27 ± 1.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>HE</td>
<td>93.33 ± 12.63&lt;sup&gt;z&lt;/sup&gt;</td>
<td>E</td>
</tr>
<tr>
<td>San Marcos: P-III</td>
<td>65/95 (68.42 ± 9.35)</td>
<td>Triclabendazole</td>
<td>15</td>
<td>6.13 ± 3.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.07 ± 2.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.00 ± 10.22&lt;sup&gt;y&lt;/sup&gt;</td>
<td>IA</td>
<td>26.67 ± 22.38&lt;sup&gt;y&lt;/sup&gt;</td>
<td>IA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Clorsulon/Ivermectin</td>
<td>15</td>
<td>8.13 ± 3.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100 ± 0.00&lt;sup&gt;y&lt;/sup&gt;</td>
<td>HE</td>
<td>100 ± 0.00&lt;sup&gt;y&lt;/sup&gt;</td>
<td>HE</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Closantel</td>
<td>15</td>
<td>7.07 ± 3.63&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100 ± 0.00&lt;sup&gt;y&lt;/sup&gt;</td>
<td>HE</td>
<td>100 ± 0.00&lt;sup&gt;y&lt;/sup&gt;</td>
<td>HE</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nitroxynil</td>
<td>15</td>
<td>5.73 ± 2.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.4 ± 0.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>93.02 ± 5.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>E</td>
<td>66.67 ± 23.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>IA</td>
</tr>
<tr>
<td>San Miguel: P-IV</td>
<td>69/124 (55.65 ± 8.74)</td>
<td>Triclabendazole</td>
<td>15</td>
<td>18.47 ± 5.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16 ± 5.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.36 ± 4.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>IA</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>IA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Clorsulon/Ivermectin</td>
<td>15</td>
<td>20.07 ± 5.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100 ± 0.00&lt;sup&gt;y&lt;/sup&gt;</td>
<td>HE</td>
<td>100 ± 0.00&lt;sup&gt;y&lt;/sup&gt;</td>
<td>HE</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Closantel</td>
<td>15</td>
<td>19.13 ± 7.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100 ± 0.00&lt;sup&gt;y&lt;/sup&gt;</td>
<td>HE</td>
<td>100 ± 0.00&lt;sup&gt;y&lt;/sup&gt;</td>
<td>HE</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nitroxynil</td>
<td>15</td>
<td>18.07 ± 8.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100 ± 0.00&lt;sup&gt;y&lt;/sup&gt;</td>
<td>HE</td>
<td>100 ± 0.00&lt;sup&gt;y&lt;/sup&gt;</td>
<td>HE</td>
</tr>
</tbody>
</table>

<sup>a</sup>EPG: Egg per gram of feces

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

<sup>x,y,z</sup>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).
Discussion

According to WAVVP, clinical trials are conducted on animals naturally infected with Fasciola hepatica, and the control period must extend for a minimum duration of 21 d or more (Wood et al., 1995). This is understood 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 evaluated in the present study, it is assured that the 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 feasible. 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.

While the WAAVP also envisions studies on antiparasitic efficacy with control groups, it was not feasible in the present study since in the Cajamarca Valley, livestock farmers 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 alongside 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.

The prevalence of Fasciola hepatica on 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 conducive to the development of the intermediate host and extensive breeding. Records of this trematode 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 closer locales, 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 the high prevalence of this trematode in the Cajamarca Valley, livestock farmers have the habit of continuously deworming their animals against F. hepatica every three or four months.
Exploiting the fact that triclabendazole is an active ingredient that acts on various stages of the trematode (Cwiklinski and Dalton, 2018), it has been indiscriminately used for a considerable period without technical considerations throughout the local livestock sector. Reports of its use even predate 1998 (Claxton et al., 1998). The average livestock owner does not conduct clinical efficacy tests of the antiparasitics they use. 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 the observations of other authors who have mentioned that resistance tends to develop when parasite control relies exclusively on the same active ingredients 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), an efficacy of 2.8% was observed; in the second (El Cortijo), 3.1%; and in a third (San Vicente), 68% (Rojas-Moncada, 2012). A year later in the same valley, in a more rigorous study, triclabendazole achieved an efficacy of 31.05% on day 14 and 13.63% on day 30 in cattle (Ortiz et al., 2013). Simultaneously, in other regions of Peru, reports of *F. hepatica* resistance to triclabendazole surfaced (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).

Local livestock owners with more resources engage in better livestock management and receive guidance from veterinarians who implement more 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, being highly effective (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).
Some authors argue 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 even reach an efficacy of 20%, 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).

Emphasis is placed on current techniques for evaluating *F. hepatica* resistance. Several diagnostic methods are available, but 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 in *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 *F. hepatica* drug resistance.

In conclusion, triclabendazole is insufficiently effective against *F. hepatica*. Antiparasitics based on clorsulon/ivermectin, closantel, or nitroxinil are alternatives showing good results in controlling this trematode in the studied zones across three provinces of the Cajamarca region. However, their use should be carefully managed, including rotation and regular clinical evaluations, to prevent antiparasitic resistance.

**Declarations**

**Acknowledgments**

To the managers of the four farms where the study was conducted for allowing and authorizing the use of the animals for the research.
Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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.

References


Coyne LA, Bellet C, Latham SM, Williams D. Providing information about triclabendazole resistance status influences farmers to change liver fluke control practices. Vet Rec 2020; 187(9):357. https://doi.org/10.1136/vr.105890


Fairweather I. Reducing the future threat from (liver) fluke: realistic prospect or quixotic fantasy? Vet Parasitol 2011; 180(1–2):133–143. https://doi.org/10.1016/j.vetpar.2011.05.034


Kelley JM, Rathinasamy V, Elliott TP, Rawlin G, Beddoe T, Stevenson MA, Spithill TW. Determination of the prevalence and intensity of Fasciola hepatica infection in dairy cattle from six irrigation regions of Victoria, South-eastern Australia, further identifying significant triclabendazole


