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ORIGINAL ARTICLE

Efficacy of the main anthelmintics used in the control of bovine flukes in warm and humid climate in Mexico

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ABSTRACT. *Fasciola hepatica* and paramphistomids are common endoparasites in cattle. Chemical control is the main method to avoid the effects of both trematodes on herd health. This study aimed to evaluate the efficacy of the most commonly used anthelmintics against trematodes (paramphistomids and *F. hepatica*) in cattle raised in a warm climate in Mexico. Fecal samples were obtained from 393 cows that were naturally infected with *F. hepatica* and paramphistomids. The fecal egg count was determined using the sedimentation technique, recording the eggs per gram of feces (EPG). Only trematode-positive cows were used to assess anthelmintics such as nitroxynil (NITROX), triclabendazole (TCBZ), fenbendazole (FBZ), rafoxanide (RAFOX), albendazole (ABZ) and oxyclozanide (OCZ). Efficacy was determined following the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines; samples were taken 15 days post-treatment to perform the fecal egg count reduction test (FECRT). According to FECRT, the efficacy of anthelmintics was 0–83.3% against paramphistomids and 51.8%–100% against *F. hepatica*. The most effective anthelmintics against *F. hepatica* were NITROX (89–100%) and RAFOX (93–100%). Triclabendazole in all its combinations (TCBZ + FBZ and ABZ) had lower efficacy in controlling paramphistomids (0–39%), but high efficacy against *F. hepatica* (59-73%). Most anthelmintics were effective against *F. hepatica*; however, control alternatives for paramphistomids require further investigation.

Keywords: *Fasciola hepatica*, paramphistomids, anthelmintic resistance, Mexico

INTRODUCTION

Livestock production is one of the main economic activities in Mexico, with annual production reaching 2.08 million tons of meat and 12.5 billion litres of milk (SIAP, 2021). Cattle production systems present great challenges, such as the warm climate as well as economic and social pressures to increase productivity (Lovarelli *et al*., 2020). Endoparasites are one of the causes of reduced livestock productivity. Among these, trematodes such as *Fasciola hepatica* and paramphistomids are the most important. Trematodes (*F. hepatica* and paramphistomids) are globally distributed and abundant in tropical and subtropical regions, where environmental conditions, including abundant streams, wetlands, and grasslands, combined with warm climates, sustain the prevalence of endemic trematodes (Mas-Coma *et al*., 2020; Olsen *et al*., 2015). These environments are ideal for the survival of snails, which are intermediate hosts in the life cycle of *F. hepatica* and paramphistomids (Fang *et al*., 2022). The sporocyst, redia, and cercaria stages develop and multiply inside the snails. These in turn become metacercariae, which, after ingestion by animals, disencyst in the small intestine and complete their life cycle by migrating to their target organ and transforming into adult trematodes (Moazeni & Ahmadi, 2016).

Fasciolosis affects the health of livestock and causes economic damage owing to low weight gain and liver damage. In addition, *F. hepatica* represents a potential danger to human health, as it is a zoonotic and re-emerging disease worldwide (Sabourin *et al*., 2018). The World Health Organization (WHO) estimates that approximately 56 million people are infected by at least one species of trematode, and up to 750 million people are at risk of infection (Elelu & Eisler, 2018). The trematodes *Paramphistomum cervi* and *Calicophoron daubneyi* have gained importance in causing paramphistomosis, an emerging disease in cattle in Europe and Southeast Asia (Červená *et al*., 2022). In cattle, *F. hepatica* infections cause liver damage (Rashid *et al*., 2019) whereas paramphistomids cause lesions in the small intestine, resulting in weight loss, reduced milk production, low fertility and, in some cases, animal mortality (Thanasuwan *et al*., 2021). It is estimated that fasciolosis causes economic losses exceeding US$3 billion per year worldwide (Elelu & Eisler, 2018). In Mexico, losses due to *F. hepatica* in untreated cattle amounted to US$130.91 per year (Rodríguez-Vivas *et al*., 2017); in addition, losses due to the use of anthelmintics to control trematodes are estimated at US$67.68 in young cattle and US$209.47 in adult cows, respectively (Villa-Mancera & Reynoso-Palomar, 2019).

The use of commercial anthelmintics is a method to control endoparasites and increase livestock productivity. However, there are multiple reports of anthelmintic resistance (AR), mainly in *F. hepatica*, to anthelmintics such as triclabendazole (TCBZ), albendazole (ABZ), closantel (CLOS), nitroxinil (NITROX), and other drugs (Novobilský & Höglund, 2015). The low efficacy of such anthelmintics will certainly compromise the future control of trematodes. Triclabendazole has been reported to have limited efficacy against *F. hepatica* in countries such as the UK (Kamaludeen *et al*., 2019). In some cases, the lack of efficacy of TCBZ leads to more applications, and the use of double doses increases the degree of AR (Kahl *et al*., 2023). Anthelmintics, such as NITROX, CLOS, TCBZ, and ABZ, are ineffective in controlling paramphistomids; however, oxyclozanide (OCZ) has proven to be effective (Nzalawahe *et al*., 2018). In Mexico, the *in vitro* efficacy of commercial anthelmintics in *F. hepatica* and paramphistomids has been studied (Jiménez-Penago *et al*., 2023) to determine the efficiency of anthelmintics. However, field trials such as FECRT are required to determine efficiency and provide recommendations to farmers. In a previous study in the same region, a low efficacy of anthelmintics against *F. hepatica* and paramphistomids was determined (Ico-Gómez *et al*., 2021); therefore, it is necessary to determine the efficacy of other anthelmintics, as many commercial mixtures have been formulated. Therefore, the objective of this study was to assess the efficacy of the most commonly used anthelmintics against paramphistomids and *F. hepatica* in cows raised in warm climates in southeastern Mexico.

MATERIALS AND METHODS

Location

The study was conducted in southeastern Mexico, where six cattle farms were visited: two located in Teapa, Tabasco, one in Villahermosa, Tabasco, two in Salto de Agua, Chiapas, and one in Escarcega, Campeche (Figure 1). The climate of the region is warm and humid with an average annual temperature of 27.8 °C and annual rainfall ranging from 2000 to 3800 mm. The region is located between 30 and 80 m.a.s.l., with wetlands, swamps and mounds predominating. The characteristic vegetation is evergreen rainforest.

Animal management

The cattle were grazing freely, and they did not receive anthelmintic treatment during the two months prior to the study. General management of the animals included annual

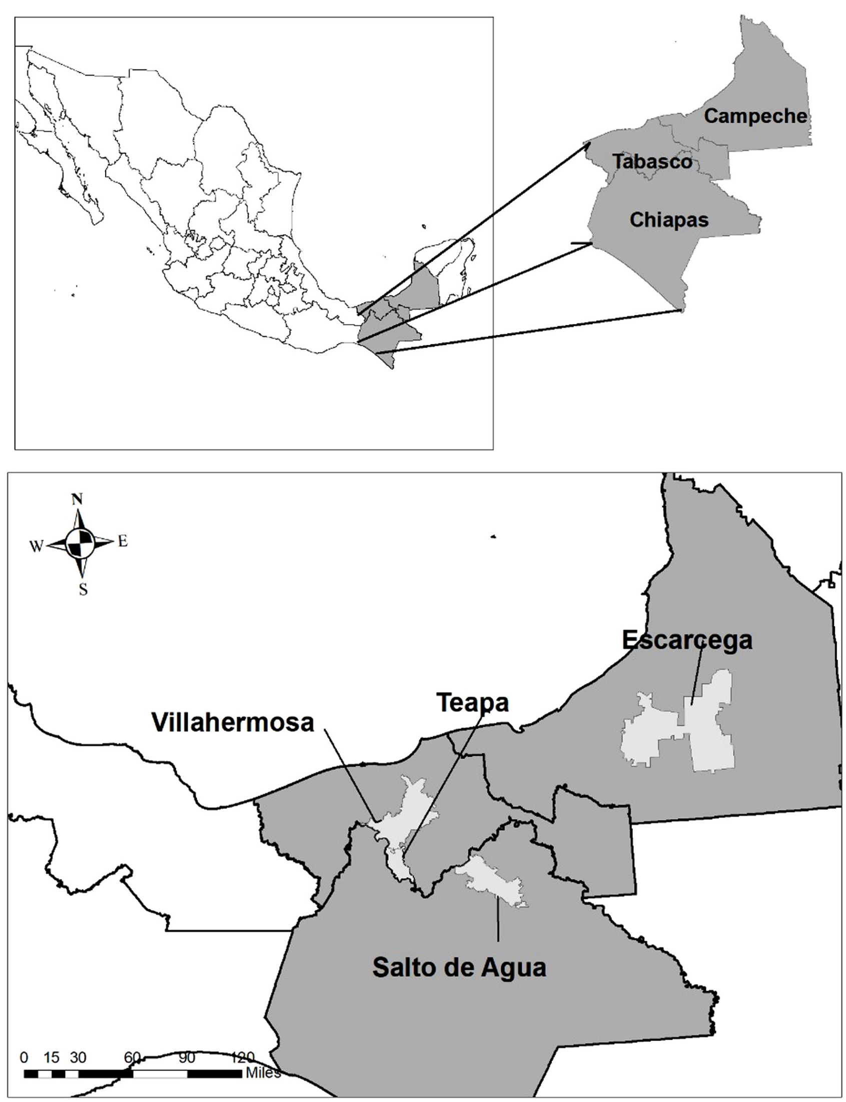

Figure 1.
Sampling area that included the states of Tabasco, Chiapas and Campeche, Mexico with a warm humid climate. The municipalities where the farms are located are highlighted in light grey.

vaccination against malignant edema, symptomatic anthrax, pasteurellosis, and bovine rabies. Anthelmintic treatment was generally performed every six months with anthelmintics such as ivermectin. The cows received mineral salts and had access to watering troughs. The ages of the cows included in the study ranged from 4 to 10 years, with different physiological states, such as lactation and pregnancy. The breeds were a cross of *Bos taurus* x *Bos indicus*.

The samples were obtained from six farms (Table 1). The largest number of cows was used in each farm to ensure an adequate number of trematode-positive animals. Fecal samples were collected directly from the rectum of the animals using polyethylene bags, which were labelled and identified with the corresponding animal number. Subsequently, the samples were transported to the laboratory for coprological analysis via sedimentation. This method allowed the counting of trematode eggs.

Coprological evaluation

From each fecal sample, 10 g of feces were weighed and mixed with 100 ml of tap water. The mixture was filtered through three sieves, the first of 50 mesh and the second

Table 1.
Sampling and deworming program for evaluating the main anthelmintics in the control of trematodes in southeastern Mexico.

Farm	State	Cattle-number	Treatments (n)	Brand name	Dose	Date sampling*
R1RSA	Chiapas	28	Control (8)			
			TCBZ + FBZ (10)	Saguaymic Plus®	12+12	30-01-21
			RAFOX (10)	Rafoxcur®	10	27-02-21
R2ASA	Chiapas	65	Control (12)			08-05-21
			NITROX (11)	Trodax® 34%	10.2	15-05-21
			TCBZ + FBZ (11)	Saguaymic Plus®	12+12	29-05-21
			RAFOX (11)	Rafoxcur®	10	12-06-21
R3RTE	Tabasco	89	Control (11)			12-08-21
			RAFOX (12)	Rafoxcur®	10	20-08-21
			ABZ + TCBZ (12)	Albendazol+Triclabendazol	12+12	06-09-21
			NITROX (12)	Trodax® 34%	10.2	20-09-21
R4FEC	Campeche	75	Control (16)			
			RAFOX (16)	Rafoxcur®	10	7-10-21
			NITROX (16)	Trodax® 34%	10.2	22-10-21
			TCBZ+FBZ (16)	Saguaymic Plus	12+12	7-11-21
R5RTE	Tabasco	55	Control (10)			
			OCZ + TCBZ + LEV + IVM (10)	Tricloxil Oral	15+12+5+0.3	24-11-21
			NITROX (10)	Trodax® 34%	10.2	15-12-21
R6AVH	Tabasco	81	Control (11)			
			OCZ + TCBZ + LEV + IVM (23)	Tricloxil Oral	15+12+5+0.3	
			TCBZ + ABZ + IVM (12)	Triclabendazole + albendazole + ivermectin	10+10+0.2	2-12-21
			NITROX (11)	Trodax® 34%	$10.20	22-12-21

TCBZ + FBZ: triclabendazole + fenbendazole (Saguaymic Plus®, Laboratorios Microsules Uruguay S.A.); RAFOX: rafoxanide (Rafoxcur®, Riverfarma S.A.); NITROX: nitroxinil (Trodax®, Boehringer Ingelheim); ABZ + TCBZ: albendazole + triclabendazole (Cheminova de Mexico S.A.); OCZ + TCBZ + LEV + IVM: oxyclozanide + triclabendazole + levamisole + ivermectin (Tricloxil Oral, Pretevet Laboratorios S.A.); TCBZ+ABZ+IVM: triclabendazole + albendazole + ivermectin (Experiencia Veterinaria®). * Sampling carried out on farms for all anthelmintics used.

of 100 mesh, both to remove large residues, and the trematode eggs retained in the 400 mesh (37 μm) were placed in a glass filled to 100 ml with tap water. After the removal of the larger residues, the sedimentation technique consisted of settling the mixture for five minutes and removing the supernatant to clean the contents. This step was repeated three times. Three drops of methylene blue were added to each sample to dye the food waste and differentiate the trematode eggs into *F. hepatica* (golden color) and paramphistomids (colorless) (Figure 2).

In a Petri dish, 2.5 mL of the contents was placed for observation using a 10× objective under an optical microscope. The sensitivity was four eggs per gram of faeces (EPG) because one Petri dish contained 0.25 g of faeces dissolved in water, so each egg found represents four eggs per gram. Another Petri dish was examined when no trematode eggs were found in the first. Average fecal egg count (FEC) was calculated from the number of positive cows on each farm.

Anthelmintic efficacy

The efficacy assessment of the most important anthelmintics used in the control of trematodes in cows included rafoxanide (RAFOX; Rafoxcur®), oxyclozanide + triclabendazole + levamisole + ivermectin (OCZ + TCBZ + LEV + IVM; Tricloxil Oral), triclabendazole + fenbendazole (TCBZ + FBZ; Saguaymic® Plus), albendazole + triclabendazole (ABZ + TCBZ; Cheminova de Mexico S.A.) nitroxynil (NITROX; Trodax® 34%) administered intramuscularly (Table 1).

All anthelmintics were used according to the manufacturer's specifications for the route of administration (intramuscular or subcutaneous) and the dose was administered according to the weight of the cows. A group of cows that did not receive anthelmintic treatment represented the control group (Table 1). Seven days before the treatment, sampling was conducted to form groups of animals. To determine the efficacy of the anthelmintics, fecal sampling was conducted 15 days post-treatment according to the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines. On some farms, the small number of positive cows prevented the testing of all anthelmintics of interest. On one farm, only traditionally used anthelmintics were tested.

The anthelmintic efficacy was evaluated by recording the trematode FEC for each anthelmintic per farm. For this purpose, only cows with four EPG were considered, because one observed egg was considered to represent a sensitivity of four eggs per gram. Fecal egg count reduction was calculated using the formula indicated by Dobson *et al*. (2012).

$$\text{Efficacy regarding post-treatment control} = 100 \times \left(1-\frac{T2}{T1}\right)$$

Where T2 represents the FEC after deworming of the treated group and T1 represents the average FEC of the control group after treatment.

On the farms, one sample was taken before treatment (-7), and another sample was taken 15 days after treatment, as indicated in the WAVVP guidelines for nematodes.

Statistical analysis

The trematode FEC data transformed to log (FEC+1) were processed and analyzed using the generalized linear model (GLM) procedure of SAS program version 9.4M5 (SAS, 2017). This information was analyzed using the following statistical model:

$$Y_{ijkl} = \mu + \text{Ƨ}_i + \text{ɗ}_l + \text{Ƨ}_i * \text{ɗ}_{ij} + \zeta(\text{Ƨ}_i)_{ik} + \varepsilon_{ijkl}$$

where Y_{ijkl} = response variable (egg count of paramphistomids and *Fasciola hepatica*); μ = general average; Ƨ_i = fixed effect of the farm (i = 1, 2, 3, 4, 5, 6); ɗ_j = sampling fixed effect (l = 1, 2); $\text{Ƨ}_i{}^* \text{ɗ}_{ij}$ = interaction between farm and sampling; $\zeta(\text{Ƨ}_i)_{ik}$ = treatment effect nested in farm; and ε_{ijkl} = experimental residue. Differences between the means of each farm were compared using the Tukey test.

RESULTS

Faecal trematode egg count

The average trematode FEC in cows was 10.3 EPG for paramphistomids and 2.6 EPG for *F. hepatica*, with differences between farms ($P \leq 0.05$). However, when only positive cows were analyzed, the average FEC (15.5 EPG) was similar between farms (Table 2). In some farms, only paramphistomids were found, as in Campeche (R4FEC farm), where the highest FEC (18.4 EPG) was observed, although there were no *F. hepatica* eggs.

In Tabasco (R5RTE farm), the highest FEC of *F. hepatica* was 35.2 EPG (Figure 2). In three farms, cows showed both trematodes (*F. hepatica* and paramphistomids) with a coinfection rate of 18.4% (R2ASA 23.1%, R3RTE 11.3%, and R5RTE 20.8%), whereas the other farms did not show coinfection because two farms did not have *F. hepatica*.

Efficacy

The efficacy of anthelmintics varied widely between farms (Table 3). In paramphistomids, the range of efficacy 15 days after anthelmintic treatment was between 0 and 83.3%, whereas in *F. hepatica*, the range was between 51.8% and 100%. For the control of paramphistomids, NITROX and RAFOX showed an efficacy greater than 67% in R2ASA and 83.3% in R6AVH farm in OCZ, but on the rest of the farms, the efficacy was less than 60%, and in R4FEC and R5RTE this anthelmintics had less than 15% efficacy. In contrast, most anthelmintics presented high efficacy against *F. hepatica*, as in the case of NITROX and RAFOX, which were 100% effective on the R3RTE farm.

Prevalence of trematodes

The prevalence of paramphistomids before the anthelmintic treatment ranged from 30.9% (17/55) to 64.0%

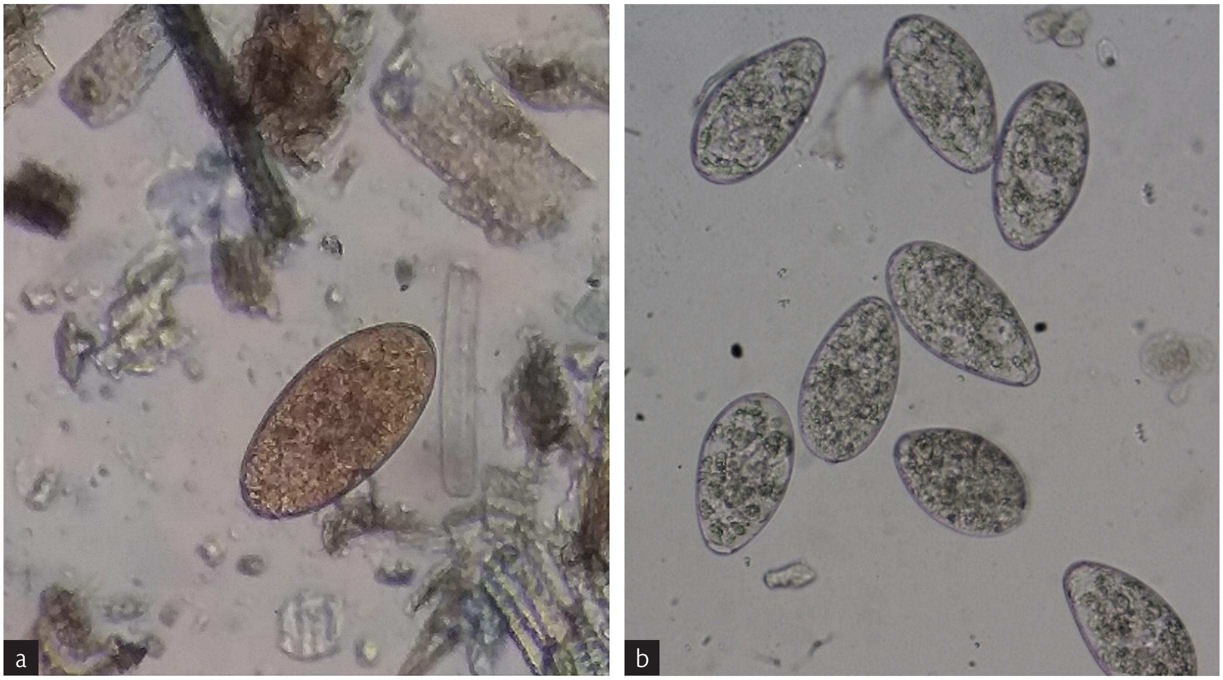

Figure 2.
Fluke eggs showing after sedimentation technique found in feces of cattle raised in southeastern Mexico: a) *Fasciola hepatica* (golden egg) and b) paramphistomids eggs (colourless).

Table 2.
Fecal egg counts of trematodes (Paramphistomids and *Fasciola hepatica*) of cattle during pretreatment (D-7) and post-anthelmintic treatment (D15) by farm.

Farm	Average per farm				Average in positive and treated cattle			
	N	D-7	N	D-15	N	D-7	N	D-15
Paramphistomids (EPG)								
R1RSA	28	1.5(0.9)^{b}	27	1.5(0.9)^{b}	7	6.1(3.1)^{a}	7	5.2(3.1)^{a}
R2ASA	65	9.0(2.3)^{ab}	57	9.2(2.5)^{ab}	27	18.7(4.7)^{a}	16	12.8(5.7)^{a}
R3RTE	80	2.9(0.8)^{b}	77	7.0(1.3)^{ab}	23	8.4(2.2)^{a}	20	13.5(3.3)^{a}
R4FEC	75	18.4(4.8)^{a}	64	13.6(3.6)^{a}	48	24.0(7.2)^{a}	32	22.5(6.8)^{a}
R5RTE	53	13.7(4.9)^{ab}	35	9.5(3.5)^{ab}	25	25.1(9.9)^{a}	12	24.8(8.6)^{a}
R6AVH	79	12.0(1.9)^{ab}	74	4.7(0.7)^{ab}	46	17.8(2.9)^{a}	28	8.9(1.5)^{a}
Fasciola hepatica (EPG)								
R1RSA	28	0.1(0.1)^{b}	27	0.1(0.1)^{b}	2	2.0(0)^{a}	1	4.0(-)^{a}
R2ASA	65	5.0(1.3)^{ab}	57	4.2(2.3)^{a}	19	15.2(3.5)^{a}	6	4.0(0.9)^{a}
R3RTE	80	2.4(0.6)^{ab}	77	2.3(0.7)^{ab}	21	7.8(1.9)^{a}	3	4.6(1.8)^{a}
R4FEC	75	0(0)^{b}	64	0(0)^{b}	0	0(0)^{a}	0	0(0)^{a}
R5RTE	53	8.9(4.0)^{a}	35	1.2(0.6)^{ab}	12	35.2(15.4)^{a}	4	8(4.0)^{a}
R6AVH	79	0(0)^{b}	74	0(0)^{b}	0	0(0)^{a}	0	0(0)^{a}

EPG: eggs per gram of feces; D-7: pretreatment sampling; D-15: sampling on day 15 post-treatment; N: number of observations; abc: different letters within a column represent significant differences ($P \leq 0.05$).

Table 3.
Percentage efficacy (95% confidence intervals), 15 days after treatment, of anthelmintic used against bovine trematodes in farms in southeastern Mexico.

Farm	Prevalence (%)		Treatment	N	Paramphistomids		*F. hepatica*	
	Paramphistomids	*F. hepatica*			FECRT (%)	95% LCL-UCL	FECRT (%)	95% LCL-UCL
R2ASA	49.3 (32/65)	27.8 (18/65)	NITROX	11	67	-30.4, 91.6	96.5	82.4, 99.3
			TCBZ+FBZ	11	3.8	-439.1, 82.8	58.8	-104.4, 91.7
			RAFOX	11	75.4	3.3, 93.8	93.3	59.5, 98.9
R3RTE	43.6 (39/89)	20.3 (18/89)	NITROX	12	7.5	-211.3, 72.5	100	
			ABZ+TCBZ	12	-138.9	-822.3, 38.1	73.1	-31.3, 93.8
			RAFOX	12	-93.5	-721.8, 54.4	100	
R4FEC	64.0 (48/75)	0 (0/75)	NITROX	16	42.3	-203.7, 89.0	-	-
			TCBZ+FBZ	16	14.5	-173.73, 73.3	-	-
			RAFOX	16	20.4	-300.3, 84.2	-	-
R5RTE	30.90 (17/55)	10.90 (6/55)	NITROX	10	15.4	-271, 80.7	88.9	19.8, 98.5
			OCZ*	10	59.8	-106.5, 92.2	96.8	63.3, 99.7
R6AVH	55.40 (45/81)	0 (0/81)	NITROX	11	58.8	11.0, 81.0	-	-
			OCZ*	23	83.3	53.0, 94.0	-	-
			TCBZ+ABZ+IVM	12	38.6	-41.1, 73.3	-	-

N: number of observations; NITROX: nitroxynil; TCBZ: triclabendazole; FBZ: fenbendazole; RAFOX: rafoxanide; ABZ: albendazole; OCZ*: oxyclozanide + triclabendazole + levamisole + ivermectin; IVM: ivermectin. FECRT: fecal egg count reduction test. LCL: Lower confidence limit, UCL: Upper confidence Limit.

(48/75). The highest prevalence of paramphistomids (64%) was observed at the R4FEC farm (Table 3). The highest prevalence of *F. hepatica* (27.8%; 18/65) was observed in Chiapas (R2ASA farm), while in R4FEC in Campeche and R6AVH in Tabasco, no positive cows were observed through sampling.

DISCUSSION

Parasitic infection by trematodes in cattle has been reported in several studies conducted in different regions of the world (González-Warleta *et al*., 2013; Khedri *et al*., 2015). Failure to control *F. hepatica* and paramphistomids has been reported in many countries, which adds risk to livestock productivity, especially in warm climates, where despite anthelmintic control, the prevalence of these endoparasites remains a persistent health problem.

Prevalence

A high prevalence of paramphistomids was recorded on all studied farms, reaching 85% in Campeche. This value is considerably high given that the climatic conditions in this region are drier than those in the other study areas, although it features muddy lands. This supports the idea that paramphistomids have a wide geographical distribution and are an emerging disease in Europe (Huson *et al*., 2017) and Southeast Asia under climatic conditions similar to those in the Mexican tropics, where the reported prevalence of paramphistomids ranges from 65% to 85%, which is comparable to that found in this study. Additionally, similar prevalences to those in some farms in Mexico have been reported in Algeria and the Netherlands (Titi *et al*., 2014). In Tabasco, Mexico, an annual prevalence of 39.10% has been reported for *Paramphistomum cervi*, a species also identified in this area (Rangel-Ruiz *et al*., 2003). Recent studies have confirmed the prevalence of paramphistomids in cattle from southeastern Mexico through coprological analyses (Ico-Gómez *et al*., 2021; Hernández-Hernández *et al*., 2023).

The trematode prevalence observed in this study may be attributed to factors such as production systems, drinking water systems, flooding around farms, grazing systems, and anthelmintic treatment, in addition to the lack of specific anthelmintics for paramphistomids, and possibly more intermediate hosts for paramphistomids (Khedri *et al*., 2015).

Nevertheless, there have been no studies on the identification and counting of snails in pastures; only the susceptibility of the genus *Lymnaea* has been evaluated in Mexico (Castro-Trejo *et al*., 1990). Further research on intermediate hosts is necessary, as this could explain the high prevalence of paramphistomids.

Before the anthelmintic treatment, the prevalence of *F. hepatica* reached 60%. Similar values have been reported in Vietnam, in Cuba during the dry season (Soca-Pérez *et al*., 2016), and in Peru, even at higher altitudes (Ticona *et al*., 2010). In Mexico, the prevalence of *F. hepatica* has been reported in slaughterhouses, with higher rates observed during periods of increased rainfall (Hernández-Guzmán *et al*., 2021), which aligns with the values found on the farms in this study. The absence of *F. hepatica* on some farms might be due to the anthelmintic control that the herd receives, but paramphistomids were identified on these farms despite deworming. This is particularly interesting, because both trematodes share a similar life cycle and intermediate host (Forstmaier *et al*., 2021). However, the presence of one of these could be due to interactions within the intermediate host (Jones *et al*., 2017) or the presence of different intermediate hosts.

Anthelmintic efficacy

In the present study, some anthelmintics, such as NITROX, RAFOX, and OCZ, alone or in combination, had low efficacy in reducing paramphistomid eggs in feces. NITROX was the most effective (85.2%) and was comparable to the 83.3% reported in adult cows from southeastern Mexico (Ico-Gómez *et al*., 2021). Although NITROX is an anthelmintic recommended to control *F. hepatica*, it affected paramphistomids, when a reduction in the fecal egg count was observed. On the other hand, OCZ is the only drug reported to be effective against paramphistomids (Hoyle *et al*., 2022), but it is not available in several countries, and is not licensed for use in the United Kingdom (Fenemore *et al*., 2021). The efficacy of OCZ reported in Tanzania has reached a FECR of 99% in bovine amphistomes (Nzalawahe *et al*., 2018). The same situation has been reported in *Calicophoron daubneyi* from dairy cattle, with an efficacy of 98–99% (Arias *et al*., 2013) while in dairy goats, the reduction was lower by 82% in the burden of immature flukes and 95.9% in the number of adult flukes (Paraud *et al*., 2009). In contrast, our results indicated 0% efficacy on some farms (R4FEC and R5RTE) and as far as 73.4% on others (R6AVH), an alarming situation because it is not an anthelmintic commonly used on farms.

The reduced efficacy of TCBZ in combination with ABZ, FBZ, and IVM (0–48%) in paramphistomids was expected because these anthelmintics are recommended for *F. hepatica* (Fairweather, 2011). Most drugs were highly effective against *F. hepatica*, as was the case for NITROX and RAFOX, in which the efficacy was 100%. NITROX represents halogenated phenols and is effective in adult and late immature flukes that migrate through the liver tissues (Omran & Ahmad, 2015). Therefore, it is a drug capable of reducing the egg count in feces and reducing the prevalence of *F. hepatica* (Romero *et al*., 2019). In Tanzania, NITROX was found to be highly effective against *Fasciola gigantica* in zebu cattle, but not effective against paramphistomids (Nzalawahe *et al*., 2018).

The high efficacy of RAFOX was surprising because it is a frequently used anthelmintic and was therefore expected to have low efficacy; however, the results indicated an FECR greater than 95–100%. In addition, OCZ, which showed 96.5% efficacy against *F. hepatica*, belongs to the group of salicylanilides, although RAFOX is most commonly used against adult flukes and immature stages (Rapic *et al*., 1988). RAFOX causes a loss of motility in adult flukes 75 min after *in vitro* treatment and results in a reduction in egg deposition between 70% and 85% at doses of 50 and 100 μg/mL, respectively (Abdel-Fatah *et al*., 2022). However, in a study on cattle, the results for ABZ and RAFOX showed an FECR between 75% and 80.6% (Shokier *et al*., 2013). Furthermore, the efficacy of RAFOX increases from 92.1% at 15 days to 97.4% at 30 days post-treatment (Zárate-Rendón *et al*., 2023). Similar results were observed in Egypt (Mostafa *et al*., 2023). RAFOX and OCZ represent an option for the control of *F. hepatica* in farms where they have a low FECR with other anthelmintics such as TCBZ.

The high efficacy of TCBZ against *F. hepatica* in sheep in Spain, England and Wales (Kamaludeen *et al*., 2019) differs from that indicated in Peru, where the efficacy was lower than 80% in cattle (Ortiz *et al*., 2013). Meanwhile in Mexico, the reported efficacy of TCBZ was 69.2% for the Mexican tropics (Ico-Gómez *et al*., 2021), while in the present study TCBZ in combination with FBZ showed an efficacy of 93%, and in combination with ABZ the efficacy was 81.3%, in contrast to results obtained against *F. hepatica* in cattle (Kouadio *et al*., 2021). Nevertheless, the efficacy of an anthelmintic cannot be generalized to all geographical areas; it must be specified by the farm and the correct use of the anthelmintic must be addressed to reduce the incidence of AR. The use of mixtures of anthelmintics is alarming, and resistance can be generated in single anthelmintics and their mixtures. In addition, access to anthelmintics is not regulated, and indiscriminate use is possible without efficacy studies and diagnoses of the prevailing parasite. There is also no adequate management of anthelmintics, which are often exposed to high temperatures, especially in hot climates; therefore, future research must consider these aspects. For paramphistomids, specific anthelmintics must be developed for this group of trematodes.

CONCLUSION

The prevalence of paramphistomids and *F. hepatica* is widespread in farms, with differences in the efficacy of anthelmintics in controlling trematodes. All anthelmintics showed an efficacy lower than 85% in paramphistomids, necessitating the search for control alternatives. Therefore, additional studies are required on this topic. In *F. hepatica*, the use of drugs combined with triclabendazole shows an early sign of AR, with the advantage that nitroxynil and rafoxanide are highly effective and can be used in anthelmintic rotation programs.

Declaration of competing interest

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

Ethical statement

The standards specified in NOM 062 ZOO 1999 were followed concerning the technical specifications for the production, care and use of laboratory animals.

Author contributions

G.J.P: Writing original draft, methodology, and data curation, R.G.G.: Writing -review & editing-, G.T.H.: Review & editing, supervision, and funding acquisition. O.M.T.C.: Writing - review & editing, methodology, and formal analysis, J.E.R.B.: Methodology, supervision, validation, visualization, D.H.S.: Writing – review & editing-, methodology, A.V,M.: Writing – review & editing- , methodology, and conceptualization.

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REFERENCES

Abdel Fatah, O. R., Arafa, W. M., Wahba, A. A. & El Dakhly, K. M. (2022). Tegumental alterations and resistance of *Fasciola gigantica* adult worms exposed to flukicides in Egypt. *Beni-Suef University Journal of Basic and Applied Sciences*, *11*, 106. https://doi.org/10.1186/s43088-022-00287-z

Arias, M. S., Sanchís, J., Francisco, I., Francisco, R., Piñeiro, P., Cazapal-Monteiro, C., Cortiñas, F. J., Suárez, J. L., Sánchez-Andrade, R. & Paz-Silva, A. (2013). The efficacy of four anthelmintics against *Calicophoron daubneyi* in naturally infected dairy cattle. *Veterinary Parasitology*, *197*(1–2), 126–129. https://doi.org/10.1016/j.vetpar.2013.06.011

Castro-Trejo, L., Garcja-Vasquez, Z. & Casildo-Nieto, J. (1990). The Susceptibility of Lymnaeid Snails to *Paramphistomum cervi* Infections in Mexico. *Veterinary Parasitology*, *35*(1-2), 157-161. https://doi.org/10.1016/0304-4017(90)90125-U

Červená, B., Anettová, L., Nosková, E., Pafčo, B., Pšenková, I., Javorská, K., Příhodová, P., Ježková, J., Václavek, P., Malát, K. & Modrý, D. (2022). The winner takes it all: dominance of *Calicophoron daubneyi* (Digenea: Paramphistomidae) among flukes in Central European beef cattle. *Parasitology*, *149*(5), 612-621. https://doi.org/10.1017/S0031182021002158

Dobson, R. J., Hosking, B. C., Jacobson, C. L., Cotter, J. L., Besier, R. B., Stein, P. A. & Reid, S. A. (2012). Preserving new anthelmintics: A simple method for estimating faecal egg count reduction test (FECRT) confidence limits when efficacy and/or nematode aggregation is high. *Veterinary Parasitology*, *186*(1–2), 79–92. https://doi.org/10.1016/j.vetpar.2011.11.049

Elelu, N. & Eisler, M. C. (2018). A review of bovine fasciolosis and other trematode infections in Nigeria. *Journal of Helminthology*, *92*(2), 128–141. https://doi.org/10.1017/S0022149X17000402

Fairweather, I. (2011). Reducing the future threat from (liver) fluke: Realistic prospect or quixotic fantasy? *Veterinary Parasitology*, *180*(1–2), 133–143. https://doi.org/10.1016/j.vetpar.2011.05.034

Fang, W., Yang, J., Wang, H. Y., Chen, S. R., Li, K. R., Liu, Y. H., Duan, Y. & Li, T. M. (2022). Exploration of animal models to study the life cycle of *Fasciola hepatica*. *Parasitology*, *149*(10), 1349–1355. https://doi.org/10.1017/S0031182022000609

Fenemore, C., Floyd, T. & Mitchell, S. (2021). Rumen Fluke in Great Britain. *Journal of Comparative Pathology*, *184*, 31–36. https://doi.org/10.1016/j.jcpa.2021.01.012

Forstmaier, T., Knubben-Schweizer, G., Strube, C., Zablotski, Y. & Wenzel, C. (2021). Rumen (*Calicophoron/paramphistomum* spp.) and liver flukes (*Fasciola hepatica*) in cattle—prevalence, distribution, and impact of management factors in Germany. *Animals*, *11*(9), 2727. https://doi.org/10.3390/ani11092727

González-Warleta, M., Lladosa, S., Castro-Hermida, J. A., Martínez-Ibeas, A. M., Conesa, D., Muñoz, F., López-Quílez, A., Manga-González, Y. & Mezo, M. (2013). Bovine paramphistomosis in Galicia (Spain): Prevalence, intensity, aetiology and geospatial distribution of the infection. *Veterinary Parasitology*, *191*(3–4), 252–263. https://doi.org/10.1016/j.vetpar.2012.09.006

Hernández-Guzmán, K., Molina-Mendoza, P., Olivares-Pérez, J., Alcalá-Canto, Y., Olmedo-Juárez, A., Córdova-Izquierdo, A. & Villa-Mancera, A. (2021). Prevalence and seasonal variation of *Fasciola hepatica* in slaughtered cattle: the role of climate and environmental factors in Mexico. *Journal of Helminthology*, *95*(e46), 1–8. https://doi.org/10.1017/S0022149X21000444

Hernández-Hernández, J. C., González-Garduño, R., Ortiz-Pérez, D. O., Villa-Mancera, A., Arias-Vázquez, M. S. & Paz-Silva, A. (2023). Prevalence of flukes (*Fasciola hepatica* and paramphistomids) in cattle in south-eastern Mexico. *Helminthologia*, *60*(2), 141–151. https://doi.org/10.2478/helm-2023-0017

Hoyle, R. C., Rose Vineer, H., Duncan, J. S., Williams, D. J. L. & Hodgkinson, J. E. (2022). A survey of sheep and/or cattle farmers in the UK shows confusion over the diagnosis and control of rumen fluke and liver fluke. *Veterinary Parasitology*, *312*, 109812. https://doi.org/10.1016/j.vetpar.2022.109812

Huson, K. M., Oliver, N. A. M. & Robinson, M. W. (2017). Paramphistomosis of Ruminants: An Emerging Parasitic Disease in Europe. *Trends in Parasitology*, *33*(11), 836–844. https://doi.org/10.1016/j.pt.2017.07.002

Ico-Gómez, R., González-Garduño, R., Ortiz-Pérez, D., Mosqueda-Gualito, J. J., Flores-Santiago, E. D. J., Sosa-Pérez, G. & Salazar-Tapia, A. A. (2021). Assessment of anthelmintic effectiveness to control *Fasciola hepatica* and paramphistome mixed infection in cattle in the humid tropics of Mexico. *Parasitology*, *148*(12), 1458-1466.https://doi.org/10.1017/S0031182021001153

Jiménez-Penago, G., González-Garduño, R., Torres-Hernández, G., Torres-Chablé, O. M., Ramírez-Bribiesca, E. & & David Hernández-Sánchez. (2023). *Fasciola hepatica* and Rumen Flukes-In Vitro Evaluation of Main Commercial Anthelmintics. *Acta Scientiae Veterinariae*, *51*,1912. https://doi.org/10.22456/1679-9216.130294

Jones, R. A., Brophy, P. M., Mitchell, E. S. & Williams, H. W. (2017). Rumen fluke (*Calicophoron daubneyi*) on Welsh farms: Prevalence, risk factors and observations on co-infection with *Fasciola hepatica*. *Parasitology*, *144*(2), 237–247. https://doi.org/10.1017/S0031182016001797

Kahl, A., von Samson-Himmelstjerna, G., Helm, C., Hodgkinson, J., Williams, D., Weiher, W., Terhalle, W., Steuber, S., Ganter, M. & Krücken, J. (2023). Efficacy of flukicides against *Fasciola hepatica* and first report of triclabendazole resistance on German sheep farms. *International Journal for Parasitology: Drugs and Drug Resistance*, *23*, 94–105. https://doi.org/10.1016/j.ijpddr.2023.11.001

Kamaludeen, J., Graham-Brown, J., Stephens, N., Miller, J., Howell, A., Beesley, N. J., Hodgkinson, J., Learmount, J. & Williams, D. (2019). Lack of efficacy of triclabendazole against *Fasciola hepatica* is present on sheep farms in three regions of England, and Wales. *Veterinary Record*, *184*(16), 502-502. https://doi.org/10.1136/vr.105209

Khedri, J., Radfar, M. H., Borji, H. & Mirzaei, M. (2015). Prevalence and Intensity of Paramphistomum Spp. In Cattle from South-Eastern Iran. *Iranian Journal of Parasitology*, *10*(2), 268–272. PMCID: PMC4522303

Kouadio, J. N., Giovanoli Evack, J., Achi, L. Y., Balmer, O., Utzinger, J., N'Goran, E. K., Bonfoh, B., Hattendorf, J. & Zinsstag, J. (2021). Efficacy of triclabendazole and albendazole against *Fasciola* spp. infection in cattle in Côte d'Ivoire: a randomised blinded trial. *Acta Tropica*, *222*, 106039. https://doi.org/10.1016/j.actatropica.2021.106039

Lovarelli, D., Bacenetti, J. & Guarino, M. (2020). A review on dairy cattle farming: Is precision livestock farming the compromise for an environmental, economic and social sustainable production? *Journal of Cleaner Production*, *262*, 121409. https://doi.org/10.1016/j.jclepro.2020.121409

Mas-Coma, S., Buchon, P., Funatsu, I. R., Angles, R., Artigas, P., Valero, M. A. & Bargues, M. D. (2020). Sheep and Cattle Reservoirs in the Highest Human Fascioliasis Hyperendemic Area: Experimental Transmission Capacity, Field Epidemiology, and Control Within a One Health Initiative in Bolivia. *Frontiers in Veterinary Science*, *7*. https://doi.org/10.3389/fvets.2020.583204

Moazeni, M. & Ahmadi, A. (2016). Controversial aspects of the life cycle of *Fasciola hepatica*. *Experimental Parasitology*, *169*, 81–89. https://doi.org/10.1016/j.exppara.2016.07.010

Mostafa, W., Abdel-Rady, A., El-Dakroury, M. F. & Felefel, W. (2023). Field trials to evaluate five fasciolicides against natural liver fluke infection in cattle and sheep in Egypt. *International Journal of Veterinary Science*, *12*(1), 76-81. https://doi.org/10.47278/journal.ijvs/2022.160

Novobilský , A. & Höglund, J. (2015). First report of closantel treatment failure against *Fasciola hepatica* in cattle. *International Journal for Parasitology: Drugs and Drug Resistance*, *5*(3), 172–177. https://doi.org/10.1016/j.ijpddr.2015.07.003

Nzalawahe, J., Hannah, R., Kassuku, A. A., Stothard, J. R., Coles, G. & Eisler, M. C. (2018). Evaluating the effectiveness of trematocides against *Fasciola gigantica* and amphistomes infections in cattle, using faecal egg count reduction tests in Iringa Rural and Arumeru Districts, Tanzania. *Parasites and Vectors*, *11*, 384. https://doi.org/10.1186/s13071-018-2965-7

Olsen, A., Frankena, K., Bødker, R., Toft, N., Thamsborg, S. M., Enemark, H. L. & Halasa, T. (2015). Prevalence, risk factors and spatial analysis of liver fluke infections in Danish cattle herds. *Parasites and Vectors*, *8*, 160. https://doi.org/10.1186/s13071-015-0773-x

Omran, E. & Ahmad, N. (2015). Effect of nitroxynil (fasciolid) on adult *Fasciola gigantica* and *Fasciola hepatica* in infected cows. *Parasitologists United Journal*, *8*(2), 107. https://doi.org/10.4103/1687-7942.175008

Ortiz, P., Scarcella, S., Cerna, C., Rosales, C., Cabrera, M., Guzmán, M., Lamenza, P. & Solana, H. (2013). Resistance of *Fasciola hepatica* against Triclabendazole in cattle in Cajamarca (Peru): A clinical trial and an in vivo efficacy test in sheep. *Veterinary Parasitology*, *195*(1–2), 118–121. https://doi.org/10.1016/j.vetpar.2013.01.001

Paraud, C., Gaudin, C., Pors, I. & Chartier, C. (2009). Efficacy of oxyclozanide against the rumen fluke *Calicophoron daubneyi* in experimentally infected goats. *Veterinary Journal*, *180*(2), 265–267. https://doi.org/10.1016/j.tvjl.2008.01.002

Rangel-Ruiz, L. J., Albores-Brahms, S. T. & Gamboa-Aguilar, J. (2003). Seasonal trends of *Paramphistomum cervi* in Tabasco, Mexico. *Veterinary Parasitology*, *116*(3), 217–222. https://doi.org/10.1016/j.vetpar.2003.07.002

Rapic, D., Dzakula, N., Sakar, D. & Richards, R. (1988). Comparative efficacy of triclabendazole, nitroxynil and rafoxanide against immature and mature *Fasciola hepatica* in naturally infected cattle. *Veterinary Record*, *122*(3), 59–62. https://doi.org/10.1136/vr.122.3.59

Rashid, M., Rashid, M. I., Akbar, H., Ahmad, L., Hassan, M. A., Ashraf, K., Saeed, K. & Gharbi, M. (2019). A systematic review on modelling approaches for economic losses studies caused by parasites and their associated diseases in cattle. *Parasitology*, *146*(2), 129–141. https://doi.org/10.1017/S0031182018001282

Rodríguez-Vivas, R. I., Grisi, L., De León, A. A. P., Villela, H. S., De Jesús Torres-Acosta, J. F., Sánchez, H. F., Salas, D. R., Cruz, R. R., Saldierna, F. & Carrasco, D. G. (2017). Potential economic impact assessment for cattle parasites in Mexico. Review. *Revista Mexicana De Ciencias Pecuarias*, *8*(1), 61–74. https://doi.org/10.22319/rmcp.v8i1.4305

Romero, J., Villaguala, C., Quiroz, F., Landaeta-Aqueveque, C., Alfaro, G. & Pérez Fernández, R. (2019). Flukicide efficacy against *Fasciola hepatica* of Triclabendazole and Nitroxynil in cattle of the central valley of Chile. *Revista Brasileira de Parasitologia Veterinaria*, *28*(1), 164–167. https://doi.org/10.1590/s1984-296120180089

Sabourin, E., Alda, P., Vázquez, A., Hurtrez-Boussès, S. & Vittecoq, M. (2018). Impact of Human Activities on Fasciolosis Transmission. *Trends in Parasitology*, *34*(10), 891–903. https://doi.org/10.1016/j.pt.2018.08.004

SAS. (2017). SAS/STAT User´s Guide (S. Inst., Ed.; Release 6.).

Shokier, K. M., Aboelhadid, S. M. & Waleed, M. A. (2013). Efficacy of five anthelmintics against a natural *Fasciola* species infection in cattle. *Beni-Suef University Journal of Basic and Applied Sciences*, *2*(1), 41–45. https://doi.org/10.1016/j.bjbas.2013.09.006

SIAP. (2021). *Servicio de Información Agroalimentaria y Pesquera*. Información Estadística. http://nube.siap.gob.mx/cierre_pecuario/

Soca-Pérez, M., Giupponi-Cardoso, P., López-Vigoa, O., Sanavria, A. & Labrada-Vázquez, A. (2016). Prevalence of *Fasciola hepatica* in grazing cows during the dry season. *Pastos y Forrajes*, *39*(4), 263–267.

Thanasuwan, S., Piratae, S. & Tankrathok, A. (2021). Prevalence of gastrointestinal parasites in cattle in Kalasin Province, Thailand. *Veterinary World*, *14*(8), 2091–2096. https://doi.org/10.14202/vetworld.2021.2091-2096

Ticona, D., Chávez, A., Casas, G., Chavera, A. & Li, O. (2010). Prevalencia de *Fasciola hepatica* en bovinos y ovinos de Vilcashuamán, Ayacucho. *Revista de Investigaciones Veterinarias del Perú*, *21*(2), 168–174.

Titi, A., Mekroud, A., El Hadi Chibat, M., Boucheikhchoukh, M., Zein-Eddine, R., Djuikwo-Teukeng, F. F., Vignoles, P., Rondelaud, D. & Dreyfuss, G. (2014). Ruminal paramphistomosis in cattle from northeastern Algeria: Prevalence, parasite burdens and species identification. *Parasite*, *21*, 50. https://doi.org/10.1051/parasite/2014041

Villa-Mancera, A. & Reynoso-Palomar, A. (2019). High prevalence, potential economic impact, and risk factors of *Fasciola hepatica* in dairy herds in tropical, dry and temperate climate regions in Mexico. *Acta Tropica*, *193*, 169–175. https://doi.org/10.1016/j.actatropica.2019.03.005

Zárate-Rendón, D. A., Briones-Montero, A., Huaraca-Oré, N. A., Veirano, G. S., Levecke, B. & Geldhof, P. (2023). Comparison of the therapeutic efficacy of five anthelmintics against natural *Fasciola hepatica* infections in dairy cattle from the Mantaro Valley, Peru. *Veterinary Parasitology: Regional Studies and Reports*, *38*, 1008027. https://doi.org/10.1016/j.vprsr.2022.100827