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Assessment of anthelmintic effectiveness to control *Fasciola hepatica* and paramphistome mixed infection in cattle in the humid tropics of Mexico

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Abstract

The objective of this study was to evaluate the effectiveness of the main anthelmintics used for the control of trematodes in cattle in an endemic area in the humid Mexican tropics. A diagnostic study was carried out in nine cattle farms to determine the prevalence of flukes through faecal examination. Only three farms with more than 20 cows positive to trematodes were chosen to determine the effectiveness of commercial anthelmintics (triclabendazole, TCBZ; ivermectin + closantel, (IVM + CLOS); IVM + clorsulon, (CLORS); nitroxynil, NITROX). The prevalence of *Fasciola hepatica* was 27.1% and 29.6% of paramphistomes. The faecal egg count of trematodes ranged from 0.0 to 12.2 eggs per gram of faeces. The highest effectiveness against *F. hepatica* was 96.7%, and 92.7% against paramphistomes. NITROX was the most effective in the control of trematodes, while other products, such as IVM + CLORS and TCBZ obtained values lower than 90%, which puts sustainable trematode control at risk. The presence of trematodes was observed on most farms, although the prevalence per herd was highly variable, which indicates that the trematodes *F. hepatica* and paramphistomes are endemic to the region and a suitable management programme is suggested to control infections caused by these parasites.

Introduction

Trematodes represent one of the main parasitic problems of the gastrointestinal tract in cattle worldwide (Merachew and Alemneh, 2020). Among the most important genera included in this class are *Clonorchis*, *Dicroelium*, *Fasciola*, *Fascioloides*, *Gigantocotyle* and various species of paramphistomes (Arias *et al*., 2011; Fairweather *et al*., 2020). These parasite infections cause economic losses due to a decrease in production by affecting the health of the animal and the increase in costs associated with treatment and control, especially when anthelmintic resistance occurs (Novobilský and Höglund, 2015; Novobilský *et al*., 2016). *Fasciola hepatica* is an important parasite because it causes a zoonotic disease, affecting animals and humans (Torgerson, 2013), widely known as fasciolosis or bovine dystomatosis. The overall estimated prevalence in Mexican herds has been high (63.56%) and the annual losses caused by increasing costs in treatment have ranged between 64.45USD and 118.47USD per cow (Villa-Mancera and Reynoso-Palomar, 2019). Losses range between 8% and 28% of meat yield, decreased milk production and liver seizure in slaughterhouses have been estimated (Torgerson, 2013; Ojeda-Robertos *et al*., 2020). In the same trematode class, the Paramphistomidae family is formed by a large group of digenetic trematodes containing several genera. Parasites of this type that have been described, particularly in ruminants are *Paramphistomum*, *Cotylophoron*, *Calicophoron*, *Ugandocotyle*, *Orthocoelium*, *Balanorchis* and *Gastrothylax*. They are commonly referred to as paramphistomes (Eduardo, 1982).

Control of this type of parasite is generally carried out with anthelmintic drugs. However, the treatment of cattle is done routinely without a previous diagnosis or knowledge of the efficacy of the product (Shokier *et al*., 2013). In the southeast of Mexico, the most commonly used fasciolicides are clorsulon (CLORS), closantel (CLOS), nitroxinil (NITROX); rafoxanide (RAFOX) and triclabendazole (TCBZ) as indicated in worldwide (Kelley *et al*., 2016). TCBZ is one of the most widely used anthelmintic drugs for the control of fasciolosis and started to be used from the 1980s onwards (Rolfe and Boray, 1987). Currently, many other anthelmintic drugs are recommended commercially; however, their efficacy towards trematodes has not been established and needs to be tested for their contribution to the development of resistance in these parasites, especially since populations of parasites resistant to flukicides have been

Table 1. Number of cattle sampled by municipality and state, in each farm

Locality	Code	Date	State	Cows (*n*)
Chapingo	RCh17Sp19	17/09/2019	Tabasco	43
Taizal	Ta24Sp19	24/09/2019	Tabasco	20
Pueblo Nuevo	PN11Oc19	11/10/2019	Chiapas	42
Galeana	Ga15Oc19	15/10/2019	Tabasco	107
Mariano Pedrero	MP29Oc19	29/10/2019	Tabasco	29
Salto de Agua	SA8Fe20	08/02/2020	Chiapas	50
Pichucalco	Pi10Fe20	10/02/2020	Chiapas	22
Pichucalco-UNACh	UN4Mr20	04/03/2020	Chiapas	50
R3Salto de Agua	SA7Mr20	07/03/2020	Chiapas	22

widely reported (Fairweather *et al*., 2020), making the control of fasciolosis difficult (Novobilský and Höglund, 2015). For this reason, the objective of this study was to evaluate the effectiveness of the main anthelmintic drugs used to control mixed fluke infection in cattle in an endemic area in the humid tropics of Mexico.

Materials and methods

Location

The study was carried out in an endemic area for flukes (*F. hepatica* and paramphistomes) from cattle, in Tabasco and Chiapas, Mexico (Table 1). The area has a warm humid climate with rain all year round, it has a precipitation range of 2500–4000 mm per year, and a temperature range that oscillates between 20°C and 28°C (CONAGUA, 2021).

Faecal egg count

To obtain the faeces, the cows in each farm were moved to a handling chute. The extraction of each sample was carried out directly from the rectum of the animals, the samples were labelled with the identification of each animal and kept at 4°C, and transported to the laboratory for coprology analysis.

Out of each faecal sample, 10 g were weighed into a glass, the contents were mixed vigorously and emptied into sieves of different sizes (12, 50, 100 and 400 mesh) placed in series; afterwards, the sample was washed with running water to remove large debris until the sample was clean. In the 400-mesh (37 μM) sieve, which corresponded to the last of the series, the contents were collected and placed in a glass, adjusting the volume to 100 mL. To read the samples, the contents of the beaker were shaken so that the sample was homogeneous and 5 mL of the contents were extracted with a pipette and placed in a Petri dish. To this sample, a drop of methylene blue was added to detect the trematode eggs. Of the 100 mL obtained, three repetitions of 5 mL each were carried out, so this was proportional to 1.5 g of faeces, and each egg found in 1.5 g represented 0.66 eggs per gram of faeces (EPG). To facilitate the reading, the faecal components were stained with methylene blue: the eggs of *F. hepatica* were yellowish and the paramphistome eggs colourless.

Species identification

The paramphistome adults recovered from the rumen at a local slaughterhouse were washed in water and fixed in formalin until processing. Sectioning specimens in the horizontal longitudinal plane was performed according to Jones (1990). After, the specimens were washed in running tap water and stained with acetic carmine dye for 15 min. Washed in distilled water and differentiated in 2% aqueous HCl solution until the body surface appeared pale pink, then again washed in distilled water. Dehydrated in a graded series of alcohols and cleared in beech wood creosote (Hycel, Mexico). Finally, they can be mounted on a slide in synthetic resins. The identification of the species was carried out with the keys proposed by Eduardo (1982) based on the morphology of the oesophagus, genital pore and acetabulum.

Anthelmintic products used

To evaluate the effectiveness of the main anthelmintic drugs used to control fluke infections in cattle, different formulations (TCBZ, IVM + CLOS, NITROX, IVM + CLORS) were tested in three farms to determine the efficacy of the main anthelmintics used in the region. In one farm ($n = 8$) TCBZ at 100 mg/mL by oral route (Endofluke, Bimeda, Dublin, Ireland,) was used. In two farms ($n = 8$, $n = 11$) IVM 2% + CLOS 10% (Closiver ADE + B12®; Andoci, Mexico) by subcutaneous route was used. IVM 1% + CLORS 10% (Biodec F; Biozoo, Jalisco, México) by subcutaneous route was used in one farm ($n = 46$). NITROX 34% (Trodax® Bovinos; Boehringer, México) in three farms was used ($n = 7$, $n = 43$, $n = 7$) and CLOS 15% (Closantil, Chinoin, Ags, México) by oral route in one farm was used ($n = 8$), by comparing FEC in treated and untreated animals (control), or by comparing FEC before and after treatment.

Effectiveness

The effectiveness of the anthelmintics was evaluated by recording the output in faeces of *F. hepatica* and paramphistome eggs for each anthelmintic by each farm. Only positive cows were considered to calculate the FEC at pretreatment by each trematode individually. In one farm it was not allowed to have a control group because the producer feared that the animals would continue to be parasitised; because of this, the effectiveness was calculated with two formulas indicated by Dobson *et al*. (2012):

$$\text{Effectiveness1 (Without control)} = 100 \times \left(1 - \frac{T2}{T1}\right)$$

$$\text{Effectiveness2 (Respect to control)} = 100 \times \left(1 - \frac{T2}{C2}\right)$$

where $T1$ and $T2$ represent the FEC before and after the treatment with anthelmintic, while $C2$ represents the FEC of the control

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Table 2. Number of positive animals (prevalence, %) to *Fasciola hepatica* and paramphistomes in cattle reared in the humid tropics of Mexico

	Positive for flukes					
Farm	*F. hepatica*	Paramphistomes	Trematodes	Mix[a]	Negative	Total
RCh17Sp19	6 (14.3)	10 (23.8)	13 (31.0)	3 (23.1)	29	42
Ta24Sp19	6 (28.6)	11 (52.4)	14 (66.7)	3 (21.4)	7	21
PN11Oc19	21 (50)	19 (45.2)	30 (71.0)	11 (37.9)	12	42
Ga15Oc19	90 (84.1)	65 (60.7)	100 (93.5)	55 (55.0)	7	107
MP29Oc19	4 (13.8)	4 (13.8)	7 (24.1)	1 (14.3)	22	29
SA8Fe20	13 (26.0)	10 (20.0)	22 (44.0)	2 (9.5)	28	50
Pi10Fe20	4 (18.2)	0 (0.0)	4 (18.2)	0 (0.0)	18	22
UN4Mr20	0 (0.0)	7 (14.0)	7 (14.0)	0 (0.0)	43	50
SA7Mr20	2 (9.1)	8 (36.4)	10 (45.5)	0 (0.0)	12	22
Average	27.1	29.6	44.9	17.9		

[a]Mixed infection was calculated as the number of animals with both species / total animals infected with any of the trematodes.

group (without deworming) on the sampling dates after deworming (Dobson *et al.*, 2012).

Sampling of animals

In the two farms from Chiapas (PN11Oc19, SA8Fe20), two post-deworming samplings were carried out: the first 15 days and the second 30 days after applying the anthelmintic. In the Tabasco farm (Ga15Oc19) only one sampling was carried out, 15 days after deworming, to observe the reduction in egg count and thus determine the effectiveness of the anthelmintics for fluke control.

Statistical analysis

The data from each farm was processed in a database and the data was later analysed using the GLM procedure of the SAS software. The comparison between the means was done by the Tukey test.

The model used was

$$Y_{ijkl} = \mu + \rho_i + \tau_j + \rho^* \tau_{ij} + \zeta(\rho)_{ik} + \varepsilon_{ijkl}$$

where Y_{ijkl} = response variable (*F. hepatica* and paramphistome egg count, egg count reduction, prevalence); μ = general mean; ρ_i = fixed effect of the farm (i = 1, 2, 3, 4); τ_j = fixed effect of the sampling (j = 1, 2, 3); $\rho^* \tau_{ij}$ = interaction between farm and sampling. $\zeta(\rho)_{ik}$ = effect of treatment nested in ranch; ε_{ijkl} = experimental error.

Results

Trematode prevalence

The prevalence per herd in the region showed high variability between farms (Table 2). The prevalence for *F. hepatica* was 27.1% and for paramphistomes 29.6%, with a range of 0–90% and 0–60.7%, respectively. The total prevalence of trematodes was 44.9%. Mixed infections were observed in 67% of the farms. Of the cattle infected with trematodes, 26.9% of the animals had mixed infections.

The species of parampisthomes found corresponded to *Cotylophoron cotylophoron* and *Paramphistomum cervi* (Fig. 1) according to their morphology. *P. cervi* has a conical body, measures 5.50 −13.23 mm long and 1.88–3.05 mm wide. Subterminal acetabulum, oesophagus 0.65–1.02 mm long, nearly straight or dorsoventrally folded. while *C. cotylophoron* is smaller, 4.82–8.93 mm long, 2.14–3.45 mm thick. Surface with papillae around oral opening, on rim of genital sucker and around acetabular opening. Sub terminal acetabulum, 1.51–2.34 mm. Pharynx 0.61–1.01 mm long.

Differential count of Fasciola hepatica *and paramphistome eggs*

The sedimentation technique combined with methylene blue staining allowed the differentiation of *F. hepatica* eggs from those of paramphistomes. The fluke FEC showed differences between farms ($P < 0.01$). Of the farms sampled, the Ga15Oc19 farm had the highest number of trematode-positive cattle and also the highest FEC of *F. hepatica* and paramphistomes (Table 3), with a mean EPG value of 12.2 for *F. hepatica* and 5.5 for paramphistomes, considering only positive animals. On the other farms, FECs were found in a range of 0.0–4.9 EPG for *F. hepatica* and from 0 to 4.4 EPG for paramphistomes.

Effectiveness

The effectiveness was very wide for both groups of trematodes. The highest effectiveness of anthelmintics was seen in farm SA8Fe20 with a range of 95.8–96.7% for *F. hepatica* and 83.8–92.3% for paramphistomes (Table 4). For *F. hepatica*, the lowest values were obtained with the mixture of IVM and CLOS in two farms and with TCBZ (46–69%), while greater effectiveness was observed with NITROX, in the range of 72–100%. In the case of IVM + CLORS, the values ranged from 79 to 90 in *F. hepatica* and from 30 to 52 in the paramphistomes.

In the sampling before treatment, cows from PN11Oc19 showed a FEC in a range of 0.25–8 EPG (Fig. 2). In this farm not all anthelmintics used were effective. On day 15 after the application of the anthelmintic, the FEC of both *F. hepatica* and paramphistomes were not reduced below the threshold (95% effectiveness). A similar situation occurred on the third sampling (day 30), when FEC in some cases decreased, but without exceeding the expected effectiveness threshold.

In the first sampling of the farm Ga15Oc19, FEC of *F. hepatica* was in a range of 8–14 EPG, while those of paramphistomes were in the range 2–7 EPG. On day15 after anthelmintic application the FEC of *F. hepatica* was reduced with the IVM + CLORS and NITROX treatments, but not with IVM + CLOS. On the other

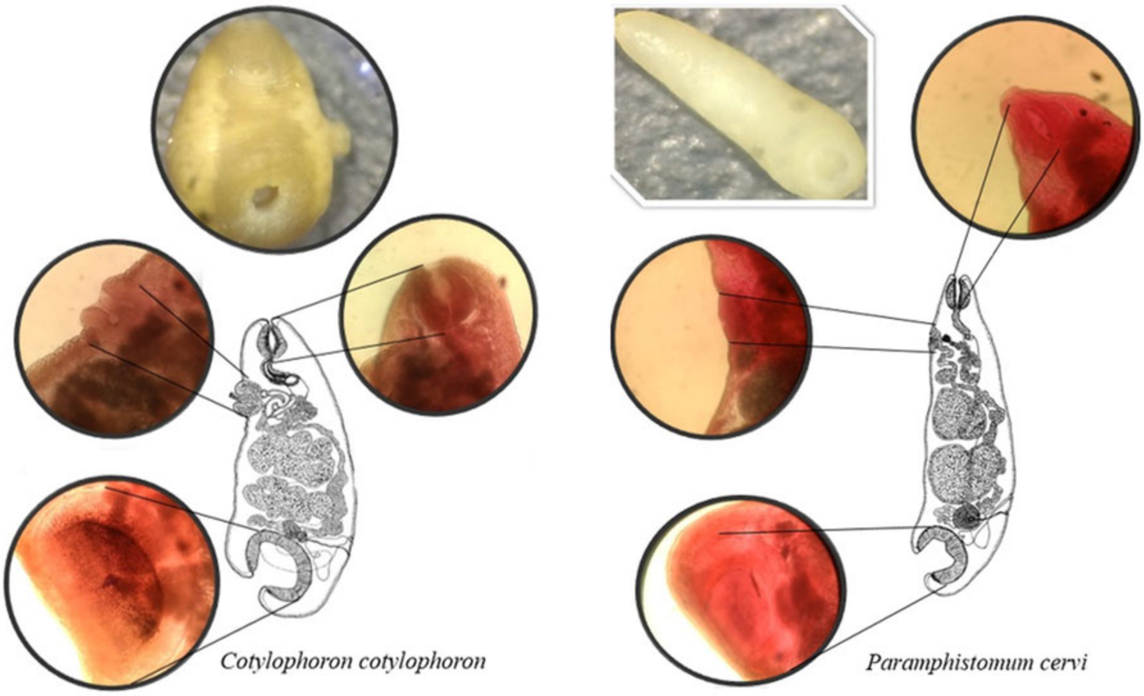

Fig. 1. Morphology of *Cotylophoron cotylophoron* (left) and *Paramphistomum cervi* (right) recovered from the rumen of bovines in Tabasco State, Mexico

Table 3. Faecal egg count mean (standard error, s.e.), by trematode type (*Fasciola hepatica* and paramphistomes) in grazing cattle in an endemic tropical region in Mexico

		Fasciola hepatica (EPG)		Paramphistomes (EPG)		Trematodes (EPG)	
Farm	*N*	All animals	Positive animals	All animals	Positive animals	All animals	Positive animals
RCh17Sp19	43	0.5 (0.2)^{b}	3.3 (0.7)^{b}	1.0 (0.4)^{bc}	4.2 (1.0)^{a}	1.4 (0.4)^{b}	4.8 (0.9)^{ab}
Ta24Sp19	20	0.8 (0.3)^{b}	2.7 (0.4)^{b}	2 (0.7)^{ab}	3.6 (1.1)^{a}	2.8 (0.8)^{b}	4.3 (0.9)^{ab}
PN11Oc19	42	2.4 (0.7)^{b}	4.9 (1.3)^{b}	1.9 (0.4)^{ab}	4.4 (0.5)^{a}	4.3 (0.8)^{b}	6.3 (1.0)^{ab}
Ga15Oc19	107	10.3 (1.2)^{a}	12.2 (1.4)^{a}	3.3 (0.5)^{a}	5.5 (0.6)^{a}	13.6 (1.5)^{a}	14.5 (1.6)^{a}
MP29Oc19	29	0.4 (0.2)^{b}	2.5 (0.5)^{b}	0.5 (0.3)^{bc}	3.5 (1.5)^{a}	0.8 (0.4)^{b}	3.4 (1.1)^{ab}
SA8Fe20	50	0.6 (0.2)^{b}	2.3 (0.2)^{b}	0.7 (0.3)^{bc}	3.4 (0.8)^{a}	1.3 (0.3)^{b}	3.1 (0.6)^{ab}
Pi10Fe20	22	0.4 (0.2)^{b}	2.0 (0.0) ^{b}	0 (0)^{c}	0 (0)^{a}	0.4 (0.2)^{b}	2.0 (0.0)^{b}
UN4Mr20	50	0 (0.0)	0 (0.0)^{b}	0.5 (0.2)^{bc}	3.7 (1.1)^{a}	0.5 (0.2)^{b}	3.7 (1.1)^{ab}
SA7Mr20	22	0.2 (0.1)^{b}	2.0^{b}	0.8 (0.3)^{bc}	2.2 (0.3)^{a}	1.0 (0.3)^{b}	2.2 (0.2)^{b}

EPG, Eggs per gram of faeces. All animals: positive and negative animals. Positive: average EPG only of positive animals. *N*: number of cows. Different letters (a,b,c) in each column represent significant differences ($P < 0.05$).

hand, the low effectiveness of the three chemicals used against paramphistomes was evident, when high FEC of these parasites was observed after anthelmintic treatment (Fig. 3).

The FEC of *F. hepatica* and paramphistomes in farm SA8Fe20 had low values at the beginning of the study (0.8–1.7 EPG). However, it was possible to observe that FEC was not decreased by these drugs and the outputs were in the range 0.2–0.8 EPG at day 15 post-treatment, while at the third sampling (day 30) the FECs for both *F. hepatica* and paramphistomes did not show statistically significant differences ($P < 0.05$) concerning days 1 and 15 of sampling (Fig. 4).

Besides, the number of fluke-positive cows after deworming ranged from 0% to 63% in *F. hepatica* with high variability between farms and in the case of paramphistomes, 13–63% of the cows were positive for these parasites (Table 5).

Discussion

Fasciolosis is a disease of great importance in animal and human health. In ruminants, *F. hepatica* reduces productivity by affecting food intake and live weight (Dorny *et al.*, 2011) and is one cause of liver seizure in slaughterhouses, causing economic losses and threatening public health (Merachew and Alemneh, 2020). Also, the immature forms of paramphistomes affect animal health during their migration to the target organ, causing severe damage to small intestine (Dorny *et al.*, 2011). Therefore, it is important to know how often mixed infections of *F. hepatica* and paramphistomes occur to take control measures. In the tropical region of Mexico, mixed infections are very common, and eggs of both trematodes were found in 26.9% of the parasitized animals using the sedimentation technique combined with methylene blue staining

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Table 4. Percentage of effectiveness of the anthelmintic used for the control of trematodes in each of the farms according to the formulas used 15 days post-treatment

		Effectiveness1 (without control)			Effectiveness2 (respect to control)		
Farm	Anthelmintic	*F. hepatica*	Paramphistomes	Trematode	*F. hepatica*	Paramphistomes	Trematode
PN11Oc19	IVM + CLOS	46.4^{b}	70.0^{a}	45.1^{b}	46.2^{b}	66.4^{ab}	41.0^{b}
	TCBZ	69.2^{b}	76.4^{a}	68.1^{b}	76.2^{b}	80.6^{ab}	73.3^{b}
	NITROX	72.2^{b}	44.0^{b}	64.0^{b}	65.5^{b}	68.1^{ab}	62.3^{b}
Ga15Oc19	IVM + CLORS	78.7^{ab}	30.5^{b}	58.3^{ab}	89.8^{a}	51.9^{b}	68.3^{ab}
	IVM + CLOS	52.7^{ab}	53.1^{b}	50.3^{ab}	64.7^{b}	68.0^{b}	60.4^{ab}
	NITROX	85.4^{ab}	55.9^{b}	75.4^{ab}	91.3^{a}	69.2^{b}	82.2^{a}
SA8Fe20	CLOS	91.7^{a}	96.2^{a}	84.4^{a}	93.8^{a}	92.8^{a}	88.9^{a}
	NITROX	100^{a}	83.3^{a}	91.1^{a}	100^{a}	91.8^{a}	94.5^{a}

Different letters (a, b) in each columns of the same variable represent statistical differences ($P > 0.05$). IVM, Ivermectin; CLOS, Closantel; CLORS, Clorsulon; TCBZ, Triclabendazole.

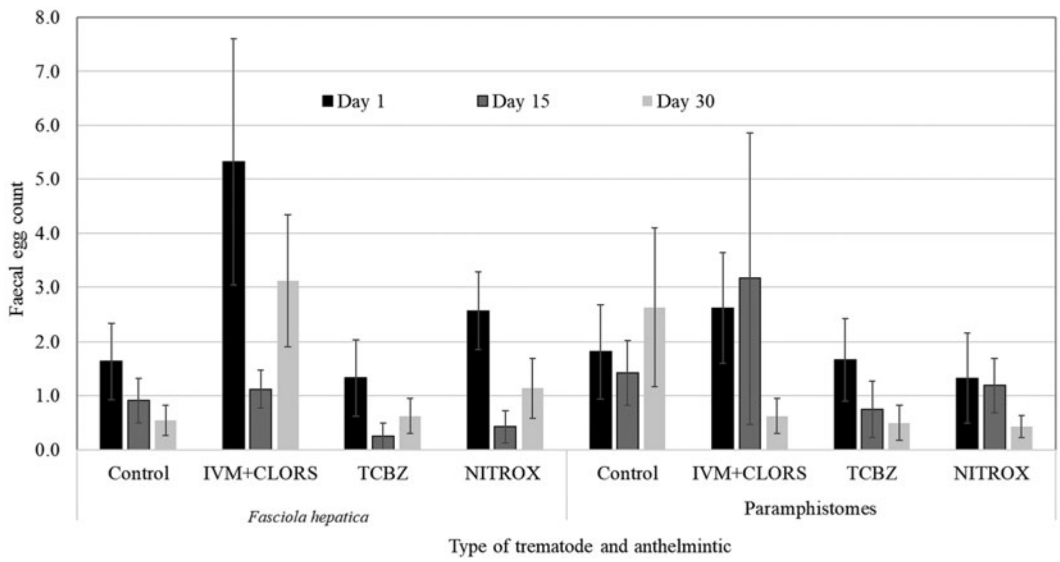

Fig. 2. Faecal count of trematode eggs (*Fasciola hepatica* and paramphistomes) by anthelmintic and sampling date in the PN11Oc19 farm. TCBZ, triclabendazole; IVM + CLORS, ivermectin + clorsulon; NITROX, nitroxynil.

that allowed the differentiation of *F. hepatica* eggs (golden colour) and those of the paramphistomes (gauzy). Of the latter, the species observed in the region were *C. cotylophoron* and *P. cervi*, however in the faeces samples, the eggs were only identified and reported as a paramphistomes, in a similar way as indicated in other study (Nurhidayah *et al.*, 2020) because it was not possible to identify eggs of each species.

The mean prevalence values of *F. hepatica* and paramphistomes (27–30%) in the study region are within the range observed in America (Paucar *et al.*, 2010; Giraldo Forero *et al.*, 2016), and also in Australia, where 39% prevalence of trematodes in cattle was determined by FEC and cELISA (Kelley *et al.*, 2020). In paramphistomes, the prevalence (29%) was at the upper limit of that reported in Europe, where the information indicates a wide range, from 6.2% to 48.8% (Ferreras *et al.*, 2014; Naranjo-Lucena *et al.*, 2018). The prevalence of trematodes had an average of 45%, this was considered as the percentage of animals that presented *F. hepatica* or paramphistomes, which turns out to be a high value, although similar values are seen in the Amazon (Pinedo *et al.*, 2010). Surprisingly, the values observed were lower than those indicated in a cold-climate country such as Ireland, where 61% of the cattle were positive for *Calicophoron daubneyi* and 68% positive for *F. hepatica*, with a co-infection between the two species of 46% (Jones *et al.*, 2017). The prevalence was highly variable among the farms studied; however, at least one species of trematode was observed in all of them. For this reason, tropical areas have been considered endemic for both *F. hepatica* and other trematodes such as paramphistomes, which represents a risk to food safety and public health (Pinilla *et al.*, 2020). In Spain, the increase in paramphistomosis has been attributed to the absence of an effective treatment against this trematode, while in Uruguay it is recommended to reduce the risk of infection by improving management to avoid exposure to this type of parasite (Sanchís *et al.*, 2013). *F. hepatica* has also been indicated to be endemic to several dairy regions in Victoria, Australia and resistance to TCBZ has contributed to the high prevalence in herds (Kelley *et al.*, 2020).

In other studies, trematodes have been identified as an economic and productive problem in bovine, in addition, the relationship of *F. hepatica* with human health makes it necessary to take control measures, as indicated in Peru, where prevalence values of 10.0 ± 2.9% are shown for *F. hepatica* and 28.4 ± 4.4% for the Paramphistomidae family (Paucar *et al.*, 2010). However, the prevalence depends on the detection method, as reported in

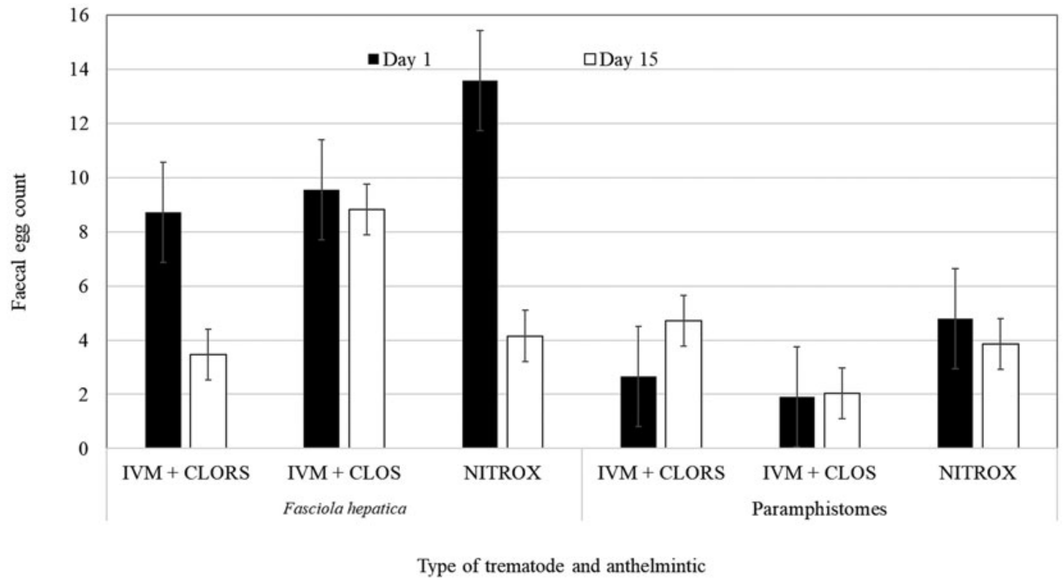

Fig. 3. Faecal count of trematode eggs (*Fasciola hepatica* and paramphistomes) by anthelmintic and sampling in the Ga15Oc19 farm. TCBZ, triclabendazole; IVM + CLOS, ivermectin + closantel; IVM + CLORS, ivermectin + clorsulon; NITROX, nitroxynil.

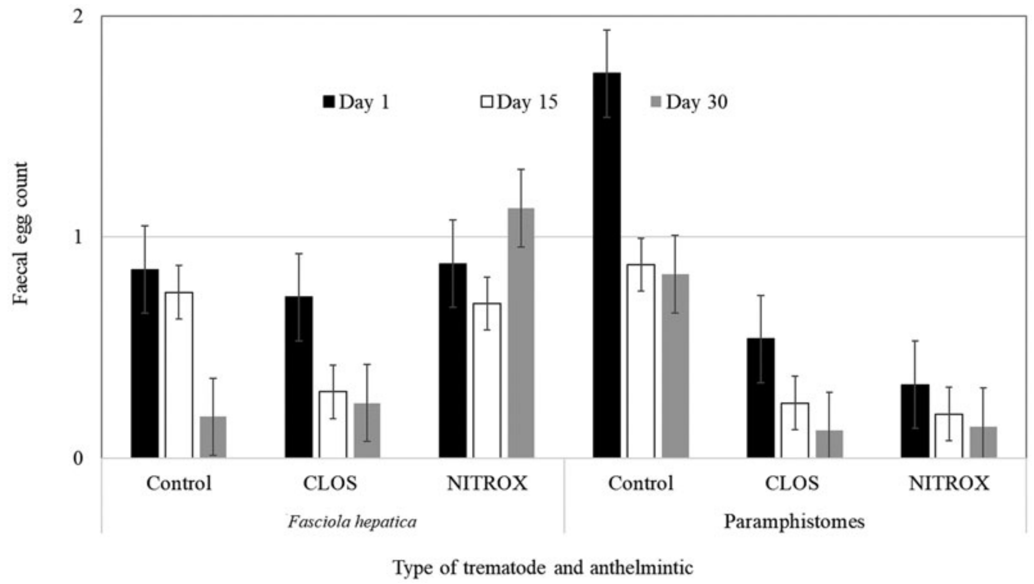

Fig. 4. Faecal egg count of trematode eggs (*Fasciola hepatica* and paramphistomes) by anthelmintic and sampling in the SA8Fe20 farm. CLOS, closantel; NITROX, nitroxynil.

Colombia, where the prevalence of *F. hepatica* was 39.4% by the presence of eggs in the bile content, 32.4% by the presence of adult parasites in the bile ducts and 15.5% by the eggs in faecal matter (Giraldo Forero *et al*., 2016). Therefore, if this information is taken into account, it can be inferred that in the study region, the prevalence would be higher than that obtained through faecal sedimentation study, and the prevalence would be 50–60%. In other regions with a warm climate, such as the province of the upper Amazon, a high prevalence (44.2 ± 4.7%) of paramphistomes has been observed. The high degree of prevalence of paramphistomes was influenced by the favourable environmental conditions present in the study area, as well as by the lack of prevention and control strategies in the local bovine population (Pinedo *et al*., 2010). Also, in buffaloes, a higher prevalence of paramphistomes (62.93%) than *F. hepatica* (16%) was indicated, probably because buffaloes are found in higher-risk environments (Nurhidayah *et al*., 2020).

The FECs reported in bovines (mean and median) in the United Kingdom have been 26.01 and 5.20 EPG, respectively, but average values of 35.6 and 14.2 EPG have been indicated during winter and summer-autumn, respectively, with a range of 0.2–220 EPG (Sargison *et al*., 2016). These values exceed those observed in the present study, in which the average reached 12.2 EPG. Although the EPG value was low in the region, the excretion per animal considering the amount of faeces eliminated daily, is very high.

Many studies about chemical control and anthelmintic resistance in *Fasciola* are been done and have considered some components as: halogenated phenol (nitroxynil) (Aktaruzzaman *et al*., 2015), salicylanides (closantel) (Malrait *et al*., 2015), benzimidazoles (triclabendazole) (Brockwell *et al*., 2014), sulphonamides (clorsulon) (Islam, 2013) and phenoxyalkanes (diamphenethides) (Elelu and Eisler, 2017). However, no products are currently licensed for the control of rumen fluke in cattle (Forbes, 2016),

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Table 5. Number of positive cows to *Fasciola hepatica* and paramphistomes (%) before and after anthelmintic treatment

			Positive to *Fasciola hepatica*			Positive to paramphistomes		
				Days post-treatment			Days post-treatment	
Farm	Treatment	*N*	Pretreatment	15	30	Pretreatment	15	30
PN11Oc19	TCBZ	7	6 (86)	1 (14)	3 (43)	6 (86)	2 (29)	2 (29)
	IVM + CLOS	8	5 (63)	5 (63)	5 (63)	6 (75)	4 (50)	3 (38)
	NITROX	8	6 (75)	2 (25)	4 (50)	3 (38)	5 (63)	3 (38)
	Control	7	4 (57)	4 (57)	3 (43)	4 (36)	5 (45)	5 (45)
Ga15Oc19	IVM + CLOS	11	10 (91)	7 (64)	-	6 (55)	4 (36)	-
	IVM + CLOR	46	41 (89)	13 (28)	-	28 (61)	29 (63)	-
	NITROX	43	39 (91)	15 (35)	-	31 (72)	24 (56)	-
SA8Fe20	CLOS	8	6 (75)	1 (13)	1 (13)	5 (63)	1 (13)	1 (13)
	NITROX	7	6 (86)	0 (0)	0 (0)	3 (43)	1 (14)	2 (29)
	Control	7	6 (86)	5 (71)	4 (57)	6 (86)	6 (86)	5 (71)

N: Number of positives cows to trematodes (*F. hepatica* or paramphistomes). IVM, Ivermectin; CLOS, Closantel; CLORS, Clorsulon; NITROX, Nitroxynil; TCBZ, Triclabendazole.

but there is documented evidence that effective drugs include the combination of oxyclozanide and levamisole (Rolfe and Boray, 1987).

In the present study the effectiveness obtained against mature stages of trematodes was worrying since the reduction values of most of them was below 90%, which is the minimum that a product must have in order not to be considered as anthelmintic resistance (Kelley *et al.*, 2020). Nitroxynil was one of the most effective anthelmintic products against *F. hepatica* and paramphistomes, and, as observed in a previous *in vitro* study, CLOS and nitroxynil showed good inhibitory effect on the development of paramphistome eggs (González-Garduño *et al.*, 2020). Unlike the results obtained in this study, the reports in *Fasciola* indicate that NITROX, oxyclozanide, CLOS and TCBZ are effective against *F. gigantica* infection at 14 post-treatments (Nzalawahe *et al.*, 2018). Additionally, the treatment with NITROX reduced the positive animals infected with *F. hepatica* (Romero *et al.*, 2019). In other study TCBZ was the most effective drug in both cattle and buffaloes with efficacy of 97.92% and 100%, respectively; Oxyclozanide and NITROX show efficacy upper to 90% at 21st day post-medication (Nzalawahe *et al.*, 2018)

There were very notable differences between farms in the effectiveness of anthelmintic. Some of the reasons include the proper management of the dosage (underdosing or overdosing) and the excessive use of these chemicals, which promotes the development of anthelmintic resistance. It is pointed out that the low effectiveness of anthelmintic occurs mainly due to frequent treatments with the same products or with products from the same family or to incorrect calculations of the dose. Such disparate results have also been reported in other studies, and the variability may be very high, as was seen in a study in Spain with dairy cattle infected with *C. daubneyi*, where a reduction of 0–26% was observed with albendazole and netobimin, but 97–99% with CLOS and oxyclosanide (Arias *et al.*, 2013). However, another study in Belgium showed that CLOS was not efficient in three herds (Malrait *et al.*, 2015) and in Sweden the first report of CLOS failure against *F. hepatica* was indicated in the same year (Novobilský and Höglund, 2015).

The high variability in the effectiveness of anthelmintic has been indicated for a long time and strong differences between cattle and sheep was indicates in CLORS against *F. hepatica*. The high effectiveness in cattle (98%) is opposite to that observed in sheep that only reached 30–60% (Fetterer *et al.*, 1985). This anthelmintic is favourite by its mode of action by inhibiting glycolysis and blocking the main energy pathway. In an *in vitro* study it was confirmed that CLORS causes severe damage to the tegument of *F. hepatica* (Meaney *et al.*, 2003). Also, results in Bangladesh confirm the high variability in the effectiveness of the products when the study shown an efficacy of 92.57% with NITROX, followed by TCBZ at 91.55% and albendazole at 84.53% (Aktaruzzaman *et al.*, 2015). In addition to the low effectiveness observed in the present study in both *F. hepatica* and paramphistomes, high variability was observed in the number of positive animals (0–64%) for both parasites after deworming, so the product to be used should be tested before it uses to control *F. hepatica* or paramphistomes to avoid the development of anthelmintic resistance. Precise recommendations should also be made based on a diagnosis made by each farm.

Regarding the use of TCBZ, it was only used in one farm because it is not a very common product in the region. Also in Nigeria this product it is not commonly used (Islam, 2013). However, in cattle in Australia, it was reported that *F. hepatica* was resistant to this product using FECRT by a coproantigen reduction test (CRT), and the results suggest that resistance is widespread in the southeast region of that country (Brockwell *et al.*, 2014) and therefore other treatment alternatives, such as new drugs or vaccines, are required. Results in Chile also indicated low effectiveness with TCBZ (Romero *et al.*, 2019). However, in Mexico, high effectiveness of TCBZ, fosfatriclaben and CLOS against *F. hepatica* has been reported in dairy cows (Rojas-Campos *et al.*, 2019); in sheep, the high fasciolicidal efficiency was observed in TCBZ (Arias-García *et al.*, 2020). Also in Bangladesh, 91.5% efficacy results were seen (Aktaruzzaman *et al.*, 2015). All these studies show high effectiveness, contrary to that obtained in this study region. In other countries, the control options depend on a wide number of products such as CLORS, NITROX, CLOS, albendazole or oxyclozanide (Kelley *et al.*, 2016). Also, combinations of products have been used to control the immature stages of trematodes. High effectiveness of moxidectin + TCBZ has been indicated, unlike mixtures ivermectin + CLOS or ivermectin + CLORS (Geurden *et al.*, 2012). The mix of ivermectin + CLORS, NITROX and combination of both, against *F. hepatica* had shown 100% efficacy. While in paramphistomes the observed effectiveness was 80% at 60 days post-treatment (Islam, 2013). This response coincides with that observed in the present study in which the greatest effectiveness of the products was in *F. hepatica*, while in paramphistomes the values were lower. However, in the opposite direction oxyclozanide was an effective trematocide against amphistomes with FECR of 99% reported in Tanzania (Nzalawahe *et al*., 2018).

In the present study, there are only results of mature states because in the case of paramphistomes the prepatent period becomes as long as 56–69 days, so more extensive studies are required to know the effect of anthelmintic in the immature stages of trematodes (Sanabria and Romero, 2008). In *F. hepatica*, the prepatent period is 12–14 weeks (Kelley *et al*., 2016), so the study only concludes on the effect of mature stages of this species.

The presence of trematodes was observed in most farms; although the prevalence was highly variable. This indicates that *F. hepatic*a and paramphistomes are endemic to the region and a suitable management programme is suggested to control infections caused by these parasites.

The most effective product in the control of trematodes was nitroxynil, while other products, such as clorsulon and triclabendazole, and mixtures of ivermectin with closantel and clorsulon, obtained values lower than 90%, which puts at risk the sustainable control of parasitosis caused by trematodes.

Data. Data availability by correspondence with the author.

Author contributions. González-Garduño and Ico-Gómez conceived and designed the study. Ortíz-Pérez and Salazar-Tapia led field work, Ico-Gómez conducted data gathering. González-Garduño and Flores-Santiago performed statistical analyses. Sosa-Pérez and Mosqueda-Gualito wrote the article.

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Conflict of interest. The authors declare there are no conflicts of interest.

Ethical standards. The procedures were carried out in accordance with the normative terms of the Mexican Official Laws, Regulations and Standards and other legal provisions are considered.

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