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DOI 10.2478/helm-2023-0017

HELMINTHOLOGIA, 60, 2: 141 – 151, 2023

Prevalence of flukes (*Fasciola hepatica* and paramphistomids) in cattle in south-eastern Mexico

J. C. HERNÁNDEZ-HERNÁNDEZ[1], R. GONZÁLEZ-GARDUÑO[1,*], D. O. ORTIZ-PÉREZ[2], A. VILLA-MANCERA[3], M. S. ARIAS-VÁZQUEZ[4], A. PAZ-SILVA[4]

[1]Unidad Regional Universitaria Sursureste, Universidad Autónoma Chapingo, km 7.5 Carr. Teapa-Vicente Guerrero, Teapa, Tabasco, México, *E-mail: *rgonzalezg@chapingo.mx*; [2]Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Chiapas, Campus Pichucalco, México; [3]Facultad de Medicina Veterinaria y Zootecnia, Benemérita Universidad Autónoma de Puebla, Tecamachalco Puebla, Mexico; [4] Facultade de Medicina Veterinaria, Universidade de Santiago de Compostela, Carvalho Calero sn. 27002, Lugo, España

Article info

Received February 21, 2023
Accepted May 15, 2023

Summary

The objective of this study was to determine the risk factors and prevalence of trematodes in south-eastern Mexico. The prevalence of trematodes was determined in 1010 bovines. The study was carried out from October 2018 (n=291) to December 2019 (n=719). Only in 2019 rumen and liver fluke eggs were differentiated. Faecal samples (n=311) were obtained from farms in southeast Mexico located in Tabasco, Chiapas and Campeche. In addition, the presence of flukes in liver and rumen from slaughtered cattle in abattoirs was recorded with a total of 408 samples. A logistic procedure was used to obtain the prevalence and the effect of main risk factors such as land physiography (flooded areas and hills), year, sex, animals' age and type of sample obtained (eggs in faeces and flukes). The general prevalence of flukes in cattle was 32.3 % in 2018 and 41.7 % in 2019. Prevalence of *F. hepatica* (liver fluke) was 18.6 % (134/719) and that of paramphistomids (rumen fluke) was 33.4 % (240/719). The infected cattle from the slaughterhouse indicated a lower prevalence of *F. hepatica* (1 %) and rumen fluke (26.7 %) than in farms detected by egg in faeces (41.8 % and 42.1 %, respectively). The physiographic zone was decisive in the presence of *F. hepatica* and rumen fluke, while sex did not represent a risk factor (P > 0.05). The environmental conditions of the Mexican southeast favour the presence of both liver and rumen fluke.

Keywords: *Fasciola hepatica*; paramphistomids; prevalence; trematodes; tropics

Introduction

In addition to the nutritional, reproductive and management aspects that limit the productivity of cattle (Vite *et al*., 2015), parasite damage represents an economic problem in livestock as they affect animal health and can even cause death of susceptible animals (Delafosse, 2022; Sargison *et al*., 2016). The adverse effects of *Fasciola hepatica* on milk production have been estimated at an approximate reduction of 1.37 – 1.78 kg of milk per cow per day, with losses between US$5,974.1 – 8,660.0 (Villa-Mancera & Reynoso-Palomar, 2019). In addition, in meat cattle production, the losses have been estimated at US$1.41 billion in Mexico, due to low weight gain and condemnation of livestock by-products (Rodríguez-Vivas *et al*., 2017). The negative effects derived from liver fluke infection affect daily weight gain, live weight and carcass weight with reductions of 9 %, 6 % and 0.6 %, respectively, although no changes have been observed in the total weight or milk production (Hayward *et al*., 2021).

* – corresponding author

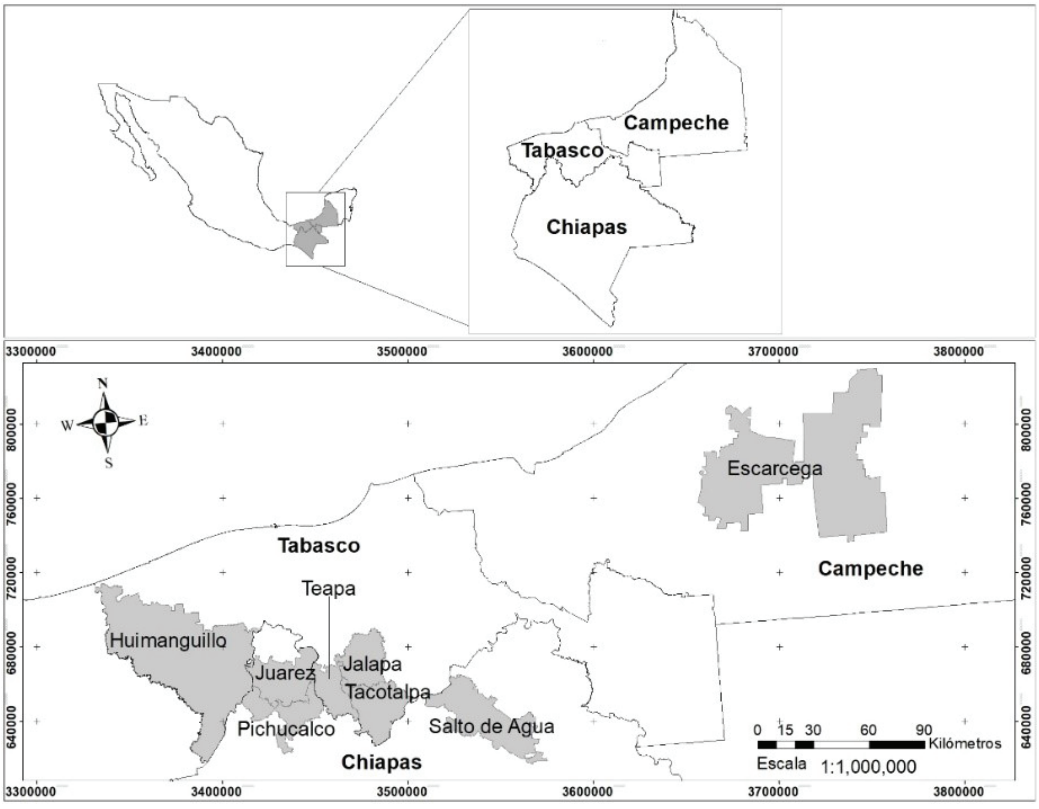

Fig. 1. Map of the study area to determine the prevalence of trematodes in southeastern Mexico.

The importance of endoparasites has been highlighted for a long time (Sardar *et al*., 2006) and it has been reported that gastrointestinal nematodes (GIN) have a high prevalence, especially in calves (Stromberg *et al*., 2015), while in adult cattle the trematodes are highly prevalent as indicated in Colombia (Pinilla *et al*., 2020). Of the flukes of veterinary importance, *Fasciola hepatica* is a cosmopolitan species with a wide presence on all continents (Jones *et al*., 2022; Nyirenda *et al*., 2019). The disease fasciolosis is caused by *F. hepatica* or *Fasciola gigantica* and together have an extensive geographic range, with a major impact on livestock (Elelu & Eisler, 2018; Naranjo-Lucena *et al*., 2018). In Mexico, the presence of *F. hepatica* in cattle has been reported for both the northeast (Munguía-Xóchihua *et al*., 2007) and southeast (Ojeda-Robertos *et al*., 2014), with highly variable prevalence reaching up to 50 % in some cases. Fasciolosis is considered an important disease because it constitutes a zoonosis and its presence has been indicated in around 70 countries with reported cases in both animals and humans (Torgerson, 2013). However, human fasciolosis has rarely been diagnosed in Mexico but a clinical report on this disease in the acute phase was reported in Puebla (Cruz y López *et al*., 2016).

In the trematode class, there is another group belonging to the Paramphistomidae family, which is composed of several genera that cause the disease called paramphistomidosis. These parasites affect the gastrointestinal tract, causing haemorrhagic inflammation of the intestines after excystation and subsequent migration of the juvenile fluke to the target organ. In acute cases the thickness of mucosa and submucosa of intestines occur (Atcheson *et al*., 2020). The presence of both species has been observed in Mexico, mainly in the tropics, where climatic conditions favour the development of gastrointestinal parasites, so the prevalence of *F. hepatica* and the existence of *Paramphistomum cervi* has been reported in Tabasco since 1989 (Rangel-Ruiz *et al*., 2003, 1999) and to date there are studies in the southern region of Mexico (González-Garduño *et al*., 2019; Ojeda-Robertos *et al*., 2020, 2014). However, the importance and high prevalence of rumen fluke have been indicated recently, a situation similar to that of many other countries that have evaluated the prevalence of this group of trematodes (Huson *et al*., 2018; Ojeda-Robertos *et al*., 2014; Ploeger *et al*., 2017).

Due to the importance of flukes in cattle health, the objective of this study was to study the risk factors and prevalence of the flukes *F. hepatica* and paramphistomids in cattle in south-eastern Mexico.

Materials and Methods

Location

The samplings were carried out on 20 cattle farms located in the municipalities of Teapa, Jalapa, Huimanguillo and Tacotalpa corresponding to the southern region of Tabasco state and in the mu-

Table 1. Number of bovines sampled per year, type of sampling and state of origin of the animals.

Year	Type of sample	State	Number of samples
2018	Faeces for determination of trematode eggs	Tabasco	193
		Chiapas	98
2019	Faeces for determination of liver and rumen fluke eggs	Tabasco	227
		Chiapas	34
		Campeche	50
	Presence of liver and rumen flukes in slaughtered cattle	Tabasco	83
		Chiapas	325
Total			1010

nicipalities of Pichucalco, Juarez and Salto de Agua corresponding to the north of the Chiapas state. Samples were also taken in Escarcega, Campeche. In addition, 14 samplings were carried out in three slaughterhouses, one located in Jalapa, Tabasco 17° 38' N and 92° 50' W, another in Juarez Chiapas located at 17° 41' N and 93° 13' W and another in Escarcega, Campeche 18° 33' N 90° 32' W (Fig. 1).

The area has a warm humid climate with rain all year round, with a precipitation range of 2000 – 2500 mm and a temperature range of 24 – 26 °C, which corresponds to a hot and humid climate with rains in summer (warm wet, Af; warm sub-humid, Am), equivalent to equatorial rainforest fully humid (Af), equatorial monsoon (Am) and equatorial savannah (warm-dry, As) (Kottek *et al*., 2006).

Cattle management

The livestock production systems were mostly for beef production and only two units were dedicated to both milk and beef production. In all farms, cattle were raised on native and introduced pastures, without supplementation, except for dairy cattle that received some type of commercial supplement. The breeds in the region are mostly crossbreeds of *Bos taurus* with *Bos indicus* and in dairy breeds, the synthetic Holstein-Friesian-Zebu breed (5/8 HF × 3/8 Zebu) was found.

Sampling

The study was carried out from August to December 2018 and in this year only the presence of flukes was determined because staining with iodine-Lugol was used to differentiate the groups, which caused an error in the differentiation and the total count was taken. In 2019 liver and rumen fluke eggs were differentiated only by the coloration of the egg. The number of samples obtained by locations are indicated in Table 1.

The farms were selected for convenience with producers, who participated as volunteers, contacted through the Livestock Producers Association. To obtain the faeces, always during the morning before 8:00 am, the cattle from each farm were moved to a handling cage where 20 – 50 g of faeces were obtained directly from the rectum of the animals in plastic gloves by veterinarians and students. Then the samples were transported to the animal health laboratory of the university for coproparasitoscopic analysis. In addition, other method to determine the prevalence was observing adult flukes, for which three slaughterhouses were visited as routine academic activities of parasitological inspection.

To report prevalence of liver and rumen fluke eggs, the sedimentation technique was used. Briefly, 10 grams of faeces were weighed and diluted in 250 ml of running water. Larger particles were removed washings the faeces through a #30 mesh sieve (0.59 mm, mont-inox) and the collected liquid was allowed to settle in beakers with 250 ml of water, then the supernatant was removed and water was added again, this procedure was carried out three times. Finally, the sediment was left in 100 ml of water and two drops of concentrated methylene blue were added. Later, the sediment from the bottom of the beaker was extracted with a pipette and the content placed in a Petri dish. The content was reviewed under a microscope (Iroscope) at 10× to watch for fluke eggs, which differed by colouring; the golden eggs corresponded to liver fluke and the transparent ones to rumen fluke (Sanabria & Romero, 2008). In addition to prevalence, the number of eggs per gram of faeces was counted in only 140 animals.

To determine the prevalence of adult trematodes, the liver, rumen and reticulum of slaughtered cattle were inspected. In addition, the liver and rumen fluke specimens were counted and the average was obtained in the positive animals. Rumen flukes were collected for their subsequent identification based on their morphology (Eduardo, 1982), for which the specimens were fixed in formalin and dehydrated. Ten specimens of each isolate were measured to discriminate species by size. Other specimens were hydrated and stained with haematoxylin-eosin and mounted on slides to differentiate them by their species characteristics (Nikander & Saari, 2007).

Statistical analysis

Two methods of prevalence were used, one with the differentiation of eggs and the other with the presence of adult flukes. The descriptive statistics using the SAS program (SAS, 2017) allowed calculating the prevalence as the number of positive cases among

the total number of animals sampled. For the analysis of the risk factors, the logistic process was used, which was carried out after the organisation of a database in Excel. The study variables were coded to adjust the response to a binomial model with two possible results: presence (1) and absence of flukes (0).

For the terrain physiography variable, two options were recorded: flood-prone areas, which were flat or slightly flat sites with clayey soils, generally flood-prone, and hillocks, which were hills or hills with slopes that did not allow water pooling (Zavala Cruz *et al*., 2016). For the sex variable, females and males were considered. For the age variable, four categories were made (calves less than one-year-old, steers from 1 to 3 years-old, young cows from 3 to 7 years-old and old cows over 7 years-old). The type of sampling consisted of evaluating the presence of fluke eggs in faeces and the presence of adult specimens in slaughtered cattle.

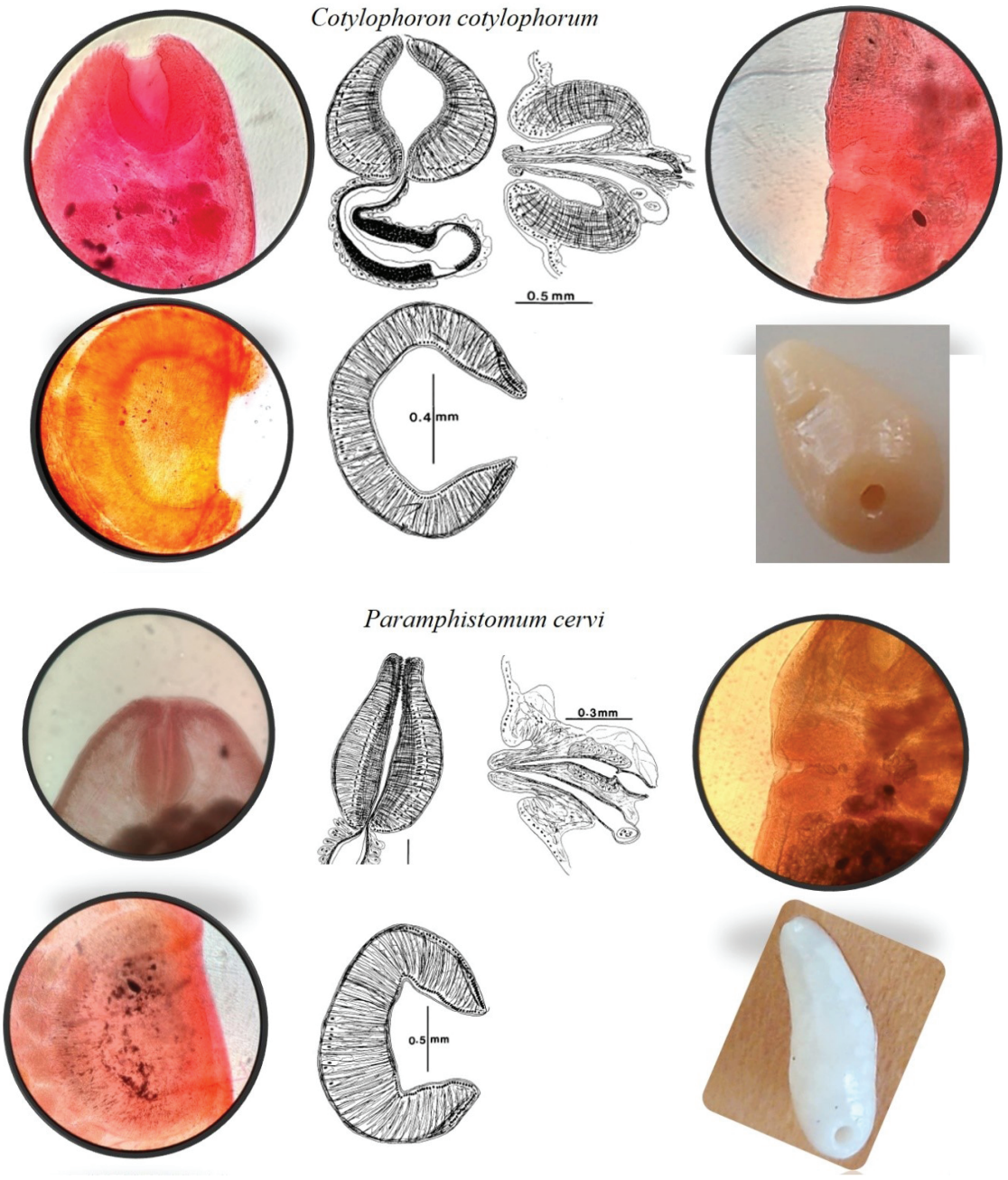

Fig. 2. Morphological identification of *Cotylophoron cotylophorum* and *Paramphistomum cervi*.

Table 2. Average faecal fluke egg (liver and rumen flukes) counts in cattle in southeastern Mexico according to sex, age and origin.

Variable	N	Mean	SE	Q1	Median	Q3
Fasciola hepatica egg per gram of faeces (epg)						
Male	11	0.2	0.2	0.0	0.0	0.7
Female	129	8.7	1.0	2.0	4.0	12.0
Steers (< 3 years)	11	0.2	0.1	0.0	0.0	0.7
Cows (> 3 years)	129	9.2	1.1	2.0	4.0	14.0
Tabasco	107	10.3	1.2	2.0	4.0	16.0
Chiapas	33	2.7	0.9	0.0	0.7	4.0
Rumen fluke egg per gram of faeces (epg)						
Male	11	0.7	0.4	0.0	0.7	1.3
Female	130	3.1	0.4	0.0	2.0	4.0
Steers (< 3 years)	11	0.3	0.1	0.0	0.0	0.7
Cows (> 3 years)	130	3.3	0.4	0.0	2.0	4.0
Tabasco	107	3.4	0.5	0.0	2.0	4.0
Chiapas	34	1.9	0.4	0.0	0.7	2.0
Trematode egg per gram of faeces (epg)						
Male	11	0.9	0.4	0.0	1.3	1.3
Female	136	11.4	1.2	2.0	4.0	15.0
Steers (< 3 years)	11	0.5	0.2	0.0	0.7	1.3
Cows (> 3 years)	136	12.0	1.3	2.0	6.0	16.0
Tabasco	107	13.7	1.5	2.0	8.0	18.0
Chiapas	40	4.5	0.9	1.3	2.0	6.5

N number of samples. SE Standard error. Q1 First quartile. Q3 Third quartile.

Ethical Approval and/or Informed Consent

All applicable international, national and institutional guidelines for the care and use of animals were followed. The procedures were in accordance with the Official Mexican Standard NOM-033-SAG/ZOO-2014 on methods to kill domestic and wild animals, and NOM-051-Z00-1995 on humane treatment in the movement of animals.

Results

The rumen fluke species identified by morphology corresponded to *Cotylophoron cotylophorum* and to *Paramphistomum cervi* (Fig. 2). In the case of *P. cervi*, the size was 7.75 ± 0.96 long and 2.75 ± 0.65 wide. Surface without papillae. Genital pore of gracile type with an absent genital sphincter and sphincter papilla. Acetabulum subterminal, from paramphistomum type. While in the case of *C. cotylophorum* the size was 6.02 ± 1.24 long and 3.40 ± 0.54 width; surface with papillae. Acetabulum subterminal, of the cotylophoron type and the terminal genitalium of cotylophoron type according to Eduardo (1985).

Faecal fluke egg counts

The faecal fluke egg counts were very low, as shown in Table 2. The highest trematode egg counts were 13.7 eggs per gram of faeces (epg) in Tabasco state. The steers under three years of age had the lowest egg counts of both liver and rumen fluke. The average of trematode eggs was 11.1 ± 14.0 epg and only 3.02 ± 4.4 epg for rumen fluke and 8.48 ± 11.04 epg for liver fluke.

Rumen fluke counts

The highest counts of adult rumen flukes corresponded to 434 specimens located in cattle in Chiapas state and only 108 in Tabasco state. The average values were 41 ± 51 specimens in the positive cattle and the values broken down by sex, age and state of origin are indicated in Figure 3.

Fluke prevalence

The prevalence of trematodes (liver and rumen fluke globally) in cattle by year was 32.3 % (94/291) to 41.7 % (300/719) in 2018 and 2019 respectively. Only in 2019 was possible to perform the differentiation of fluke eggs, obtaining a prevalence of liver fluke of 18.6 % (134/719) and of rumen fluke was 33.4 % (240/719).

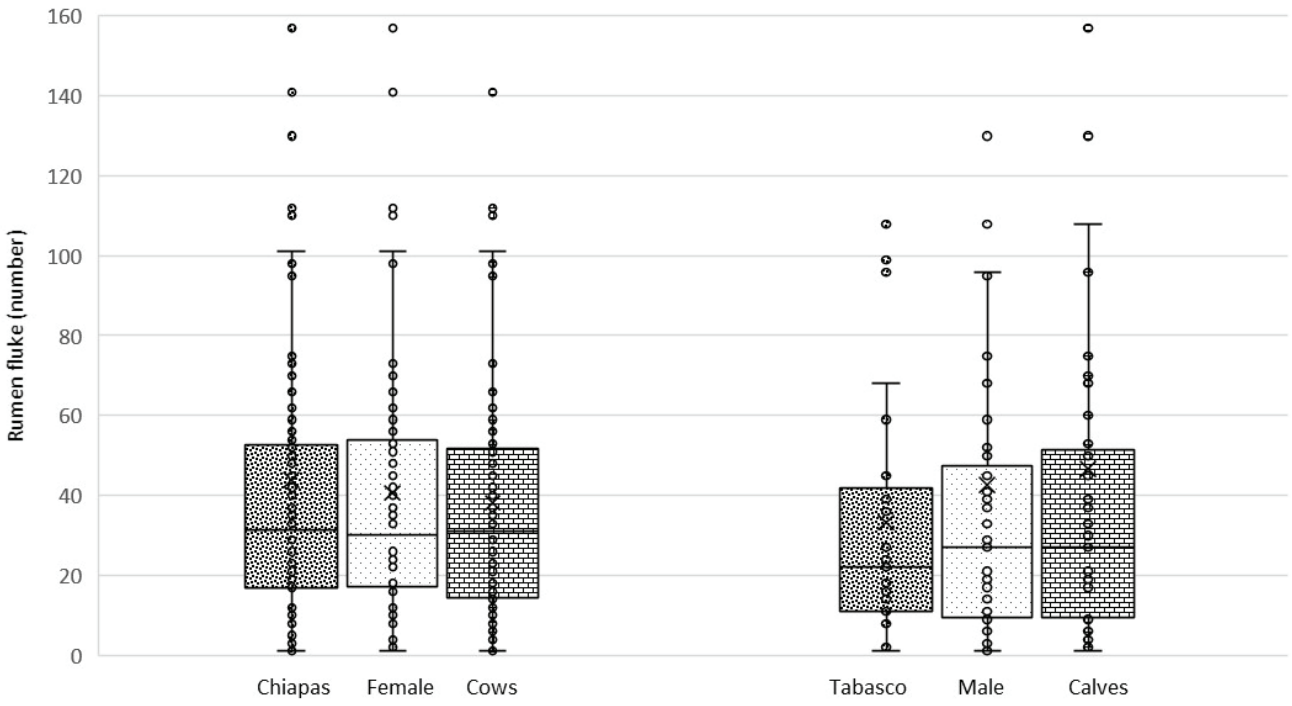

Fig 3. Fluke counts after cattle slaughter in southeastern Mexico by origin, sex and age.

The prevalence by origin of sample (faeces or adult trematode in slaughterhouse), physiographic zone, locality, sex and age of cattle only for differentiated eggs in 2019 are presented in Table 3.

The prevalence of *F. hepatica* calculated from the slaughterhouse was lower than obtained by the presence of eggs in faeces. Females presented higher prevalence than males and the age also influences the prevalence with higher prevalence in cows than steers.

Of the risk factors analysed, the type of sample was decisive in the diagnosis of trematodes and a small number of cases were observed at the slaughterhouse respect to prevalence by egg in faeces. In addition, physiographic conditions and the origin of the animal were conclusive in the presence of this parasite. Sex and age were factors that did not affect the presence of flukes (Table 4).

In the case of rumen fluke, no differences were observed in the type of sample, so the diagnosis by eggs in faeces and flukes in rumen showed similar prevalence. No differences were found in sex (Table 5) and there were also no differences in liver flukes in relation to age.

Discussion

In the present study, the prevalence of trematodes in Tabasco, Chiapas and Campeche states in Mexico reached 36 – 41 %, while for *F. hepatica* it was only 18.6 % and for rumen flukes 33.4 %. These values are at the same level as those indicated in previous studies in this area in which a prevalence of 29 % is reported for liver fluke and 27.6 % for paramphistomids, the latter with a range of 27 to 50 % (González-Garduño *et al*., 2020; Ico-Gómez *et al*., 2021). The studies with liver fluke in other countries of the American continent such as Colombia indicated 20.5 % of coprological prevalence and 41.5 % of seroprevalence (Pinilla *et al*., 2020) and in Peru a prevalence of 59 % was reported (Julon *et al*., 2020). In addition, in Argentina, a prevalence range of 40 – 80 % has been documented (Kleiman *et al*., 2007), while in Brazil, the rumen fluke prevalence of 26 % was indicated in a study from 1982 (Mattos & Ueno, 1996) and a similar prevalence was indicated in Venezuela (Gauta *et al*., 2011).

Paramphistomids also have a wide dispersion and in the last two decades reports have increased in various countries (Sanabria & Romero, 2008) even in Europe, where it is considered an emerging disease (Huson *et al*., 2017). Generally, high importance has been attributed to liver fluke due to its ability to infect different species of mammals, including humans, and there are generally more studies than those of rumen fluke, although the coexistence of both flukes is the result population dynamics of hosts influenced by environmental factors (Dreyfuss *et al*., 2014; Naranjo-Lucena *et al*., 2018).

Of the two rumen fluke species found in the region, *P. cervi* has been cited in previous studies in the same region, while *C. cotylophorum* has been described morphologically and recently in Camagüey, Cuba, was identified molecularly (León Companioni *et al*., 2020). In Mexico, is important that subsequent studies perform molecular analysis because is necessary to discriminate be-

Table 3. Prevalence of flukes in cattle in a humid warm climate of Mexico by study variable.

Risk factor			*Fasciola hepatica*	Rumen fluke	Trematodes	
		N	Positives (%)	Positives (%)	N	Positives (%)
Year						
	2018*	291	-	-	291	94 (32.3)
	2019	719	134 (18.6)	240 (33.4)	719	300 (41.7)
Type of sample						
	Eggs in faeces	311	130 (41.8)	131 (42.1)	602	281 (46.7)
	Flukes	408	4 (1.0)	109 (26.7)	408	113 (27.7)
Physiographic zone						
	Flood-prone areas	278	117 (42.1)	138 (49.6)	321	216 (67.3)
	Hillocks	441	17 (3.9)	102 (23.1)	689	178 (25.8)
Sex						
	Male	204	1 (0.5)	52 (25.5)	212	52 (24.5)
	Female	515	133 (25.8)	188 (36.5)	798	342 (42.9)
Age						
	Steers >1<3 years	187	1 (0.5)	45 (24.1)	254	57 (22.4)
	Young cows (<7)	424	131 (30.9)	160 (37.7)	623	289 (46.4)
	Old cows (> 7y)	108	2 (1.8)	35 (32.4)	133	48 (36.1)
State						
	Tabasco	310	109 (35.2)	119 (38.4)	503	207 (41.2)
	Chiapas	359	25 (7.0)	102 (28.4)	457	168 (36.8)
	Campeche	50	0 (0.0)	19 (38.0)	50	19 (38.0)

N. Total number of animals sampled. * In 2018 the prevalence was only for trematodes eggs.

tween the different species that have been reported in the region (González-Garduño *et al*., 2020).

The average counts of rumen flukes obtained in Mexico were lower (41 adult specimens) than those indicated in Iran (100 – 200 adult flukes) in slaughterhouse studies (Khedri *et al*., 2015). Additionally, in Spain, highest values were recorded (165 – 300 adult specimens) (González-Warleta *et al*., 2013). In the case of faecal egg counts, in France was observed that 19 % of bovines excreted more than 200 epg of rumen fluke (Delafosse, 2022), values higher than the average count obtained in the present study. In Spain, the mean values in excretion are close to 20 epg (González-Warleta *et al*., 2013). However, for *F. hepatica* in cattle from Argentina (Moriena *et al*., 2004), a small number of trematode eggs with values very similar to those indicated in the present study was reported.

The prevalence of both flukes (liver and rumen flukes) depended on the type of diagnostic test performed and with sedimentation technique the highest prevalence was observed. Although it was not possible to perform diagnostic tests in blood, seroprevalence is more sensitive and therefore, of a higher value than the detection of eggs in faeces (40 % vs 20 %), as indicated by a study on *F. hepatica* in cattle in Colombia (Pinilla *et al*., 2020). The results of the faecal egg count in the region showed that 18.6 % of the adult animals were infected. However, it could be a higher percentage since some were not detected with this technique, which can have 59 to 68 % sensitivity (Carneiro *et al*., 2018). In slaughterhouse studies show a higher prevalence for *F. hepatica* due to the presence of eggs in the bile content (39.4 %) and lower prevalence due to the presence of adult parasites in the bile ducts (32.4 %) and the egg count (15.5 %) in faeces (Giraldo Forero *et al*., 2016). However, studies in slaughterhouses show a great variability in results with values as low as 12 % and up to 30 % with the condemnation of livers due to the presence of *F. hepatica* (Hernández-Guzmán *et al*., 2021). The low prevalence of *F. hepatica* (1 %) in the slaughterhouses of the present study can be associated with the fact that they were the result of samplings carried out at fixed times and not of the systematic registration of all animals that entered the slaughterhouses. In addition, many of the animals destined for slaughter have come from feedlots where they were previously dewormed and therefore, less likely to carry adult parasites. In the case of rumen fluke, the diagnosis in faeces (42 %) was higher than prevalence of adult parasites in the rumen of sacrificed animals (26.7 %), as previously indicated.

Table 4. Odds ratio of the study variables that affect the presence of trematodes (*Fasciola hepatica* and rumen fluke) in cattle in a hot humid climate.

Risk factor	B	Standard error	Wald	Odds ratio	95% CI
Constant	-3.9	0.8	22.45		
Type of sample					
Fluke	Baseline			1.00	
Eggs in faeces	1.19	0.49	6.0	3.3*	1.27-8.53
Physiographic zone					
Hillocks	Baseline			1.00	
Flood-prone areas	2.48	0.37	44.7	11.98**	5.78-24.82
Sex					
Female	Baseline			1.00	
Male	-0.35	0.49	0.52	0.71^{ns}	0.27-1.83
Age					
Old cows (> 7y)	Baseline			1.00	
Steers >1<3 years	-0.81	0.53	2.33	0.44^{ns}	0.16-1.26
Young cows (<7)	-0.24	0.42	0.32	0.78^{ns}	0.35-1.79
State					
Campeche	Baseline			1.00	
Tabasco	1.98	0.61	10.46	7.27**	2.19-24.21
Chiapas	3.29	0.75	18.90	26.88**	6.09-118.51

**P-value highly significant <0.01, *significant <0.05, ^{ns}not significant >0.05, CI confidence interval

Of the risk factors, the land physiography (landform including topography, water and soil) is very important in the presence of parasitic diseases, both for fasciolosis and for paramphistomosis. In the farms located in flat areas prone to flooding with soils with a high proportion of clay, the probabilities of occurrence of *F. hepatica* were 16 times higher than in hilly areas. In the case of rumen flukes, there was a five times greater probability of infection in flood-prone areas than in the hills. Environmental conditions have been considered as one of the main factors affecting the distribution of *F. hepatica* (Hernández-Guzmán *et al*., 2021), among which the type of soil, precipitation and altitude stand out (Jiménez-Rocha *et al*., 2017), as confirmed by a study in Bangladesh (Khan *et al*., 2017). These factors affect the distribution of both liver and rumen flukes and their intermediate host, snails of species as *Galba truncatula* (Jones *et al*., 2022) *Lymnaea glabra* (Dreyfuss *et al*., 2014; Naranjo-Lucena *et al*., 2018) and in Mexico the presence of *Fossaria humilis* and *F. bulimoides* (Cruz-Mendoza *et al*., 2004) is related with the presence of *F. hepatica*. However, for rumen fluke it is necessary to determine the intermediate host species related to the highest occurrence. In addition to environmental conditions, another author attributes the high prevalence of trematodes to a lack of prevention and control strategies in the bovine population (Pinedo *et al*., 2010).

In the place of origin, differences were observed in the prevalence of both trematodes, which is the result of multiple factors that affect the presence of the parasite, as suggested by studies that address the diagnosis of these trematodes (Hernández-Guzmán *et al*., 2021; Jiménez-Rocha *et al*., 2017; Jones *et al*., 2022; Naranjo-Lucena *et al*., 2018).

Females presented higher prevalence than males because this last receive different health and nutritional management that females, especially when they are destined for slaughter. In relation to age, the results of this study indicate a higher prevalence in adult animals, which is consistent with the study carried out by Moriena *et al*. (2004) in Argentina, whose results indicate that the prevalence rate increased in direct proportion to the animals' age. Additionally, in Colombia, bovines older than two years of age had a five times greater risk of presenting these parasites than younger animals (Pinilla *et al*., 2020). The low prevalence found in young cattle has been associated in principle with the long prepatent period of *F. hepatica* (12 weeks), so the presence of eggs in faeces could be higher in adult animals, due to the probability of infection increases in grazing (Livia-Córdova *et al*., 2021). In addition, in the case of rumen fluke, the effectiveness of anthelmintics is much lower than in the case of *F. hepatica* (Ico-Gómez *et al*., 2021). It may be one of the reasons why the prevalence results are higher

Table 5. Odds ratio of the study variables that affect the presence of liver and rumen fluke in cattle in a hot humid climate.

	Fasciola hepatica					Rumen fluke				
Risk factor	B	SE	Wald	Odds ratio	95% CI	B	SE	Wald	Odds ratio	95% CI
Constant	-21.31	261.6	0.006			-2.05	0.71	8.23		
Type of sample										
Fluke	Baseline			1.0					1.0	
Eggs in faeces	2.8	0.67	17.4	16.35**	4.4-60.8	0.43	0.45	0.88	1.53^{ns}	0.63-3.73
Physiographic zone										
Hillocks	Baseline			1.0					1.00	
Flood-prone areas	2.8	0.38	50.35	15.76**	7.36-33.76	1.6	0.36	19.15	4.94**	2.41-10.1
Sex										
Female	Baseline			1.0					1.0	
Male	-2.8	1.28	4.92	0.058*	0.005-0.72	-0.09	0.44	0.05	0.91^{ns}	0.38-2.17
Age										
Old cows (> 7y)	Baseline			1.0					1.0	
Steers	0.12	1.61	0.05	1.13^{ns}	0.05-26.67	-0.59	0.52	1.28	0.55^{ns}	0.19-1.54
Young cows (<7)	0.96	0.98	0.96	2.6*	1.38-17.7	-0.45	0.43	1.07	0.64^{ns}	0.27-1.49
State										
Campeche$^{\Omega}$	-	-	-	-	-	Baseline			1.00	
Chiapas	Baseline			1.0		1.33	0.65	4.2	3.78*	1.06-13.5
Tabasco	-0.31	0.6	0.29	0.73^{ns}	0.24-2.25	0.68	0.51	1.8	1.98^{ns}	0.72-5.4

**P-value highly significant <0.01, *significant <0.05, ^{ns}not significant >0.05, CI confidence intervals. $^{\Omega}$ Liver fluke was not observed in Campeche.

for this group of parasites. In addition, other studies should be conducted to determine the influence of the host on the prevalence of paramphistomidosis.

Conflict of Interest

Authors state no conflict of interest.

Authors Contribution

Study conception and design: RGG, and JHH; sample collection and analyses: JHH, and DOOP; Data analysis and interpretation of results: AVM and MSAV; manuscript preparation: RGG and APS; Final review of the draft: MSAV, APS and RGG.

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