Journal of Helminthology

cambridge.org/jhl

Evaluation of anthelmintic drugs against egg development of rumen flukes recovered from cattle raised in the humid tropics of Mexico

R. González-Garduño[1], D.O. Ortiz-Pérez[2], L. Alegría-Jiménez[2], O.M. Torres-Chable[3], A.A. Cruz-Tamayo[4] and C.V. Zaragoza-Vera[3]

[1]Unidad Regional Universitaria Sursureste, Universidad Autónoma Chapingo, Km 7.5 Carr. Teapa-Vicente Guerrero, Teapa, Tabasco, Mexico; [2]Facultad De Medicina Veterinaria y Zootecnia, Extensión Pichucalco, Universidad Autónoma de Chiapas, Libramiento de la Ciudad, Boulevard Universitario S/N. Col, Napaná, Chiapas, Mexico; [3]Laboratorio de Enfermedades Tropicales y Transmitidas por Vectores, División Académica de Ciencias Agropecuarias, Universidad Juárez Autónoma de Tabasco, Km. 25, Carretera Villahermosa-Teapa, R/a La Huasteca 2a Seccion, CP, 86298 Villahermosa, Tabasco, Mexico and [4]Escuela Superior de Ciencias Agropecuarias, Universidad Autónoma de Campeche, Campeche, Mexico

Research Paper

Cite this article: González-Garduño R, Ortiz-Pérez DO, Alegría-Jiménez L, Torres-Chable OM, Cruz-Tamayo AA, Zaragoza-Vera CV (2020). Evaluation of anthelmintic drugs against egg development of rumen flukes recovered from cattle raised in the humid tropics of Mexico. *Journal of Helminthology* **94**, e177, 1–7. https://doi.org/10.1017/S0022149X20000607

Received: 11 May 2020
Accepted: 8 July 2020

Key words:
Paramphistomidae; hatching; anthelmintics; rumen fluke

Author for correspondence:
O.M. Torres-Chable, E-mail: oswaldo.torres@ujat.mx

Abstract

Paramphistomosis is a parasitic disease endemic in ruminants nearly worldwide. In the present study, an *in vitro* screening of the main anthelmintics used in Mexico was carried out to determine the mean lethal dose for rumen fluke eggs from cattle in a humid, warm region. Rumen flukes were obtained from cattle slaughtered in the states of Tabasco and Chiapas in Mexico. Eggs were collected using a 37-µm sieve and quantified. Then, an *in vitro* incubation study was performed: 100 eggs were placed into the wells of polystyrene microtiter plates. Anthelmintic products were tested on the eggs at concentrations ranging from 0.0015 to 3.0 mg/ml for rafoxanide, 0.0025 to 10.20 mg/ml for nitroxinil and 0.0015 to 3 mg/ml for closantel to determine the median lethal dose (LD_{50}) and maximum lethal dose (LD_{99}). A control group (water) was included in each plate. Three different species of rumen flukes (*Calicophoron brothriophoron*, *Calicophoron clavula* and *Paramphistomum cervi*) belonging to five isolates were identified. Nitroxinil had the highest efficacy against rumen fluke eggs, with an LD_{50} of 0.11 to 65 µg/ml, whereas rafoxanide showed the lowest efficacy with an LD_{50} ranging from 500 to 1713 µg/ml. Closantel showed high variability in the LD_{50} among the different analysed isolates (17 to 122 µg/ml). The evaluated flukicidal drugs presented differential efficacy against the development of rumen fluke eggs. The efficacy of the drugs will vary depending on the geographical area of origin of the animals.

Introduction

Rumen flukes are trematodes that inhabit the reticulum and rumen of several domestic and wild ruminants. These parasites have recently been recognized as a significant threat to livestock, causing significant damage to their ruminant hosts and, consequently, economic losses (Huson *et al.*, 2017). They have a heteroxenous life cycle that involves freshwater snails of various families such as Bulinidae, Lymnaeidae and Planorbidae as intermediate hosts (Rojo-Vázquez *et al.*, 2012). First, the eggs hatch in the environment, and the larvae that emerge (ciliated miracidia) infect snails, where they develop until reaching the cercaria stage. The cercariae then emerge from snails, encyst on vegetation and develop into metacercariae, which can be ingested during grazing by ruminants, which are their definitive hosts. After ingestion, the immature parasites first establish in the small intestine, but later cause significant damage as they move from the small intestine to the reticulum or rumen (Millar *et al.*, 2012; Pavan Kumar *et al.*, 2016).

Infections involving rumen flukes can cause fibrinonecrotic enteritis (Tehrani *et al.*, 2015), intestinal haemorrhages, anaemia, recurrent ruminal tympani, diarrhoea, weakness and even mortality in heavy infections, especially in young animals and small ruminants (Millar *et al.*, 2012).

Despite damages caused by rumen flukes in their ruminant hosts, these infections were considered of little importance, especially when compared with *Fasciola hepatica* infections. Only recently have these flukes been recognized as an important cause of economic losses in ruminant production systems, mainly from the UK (Malrait *et al.*, 2015; Sargison *et al.*, 2016).

The presence of rumen fluke infections in ruminants has been widely reported across several European countries such as the UK (Jones *et al.*, 2017), Ireland (Toolan *et al.*, 2015) and the Netherlands (Ploeger *et al.*, 2017), and on other continents such as Oceania (Cauquil *et al.*,

CAMBRIDGE UNIVERSITY PRESS

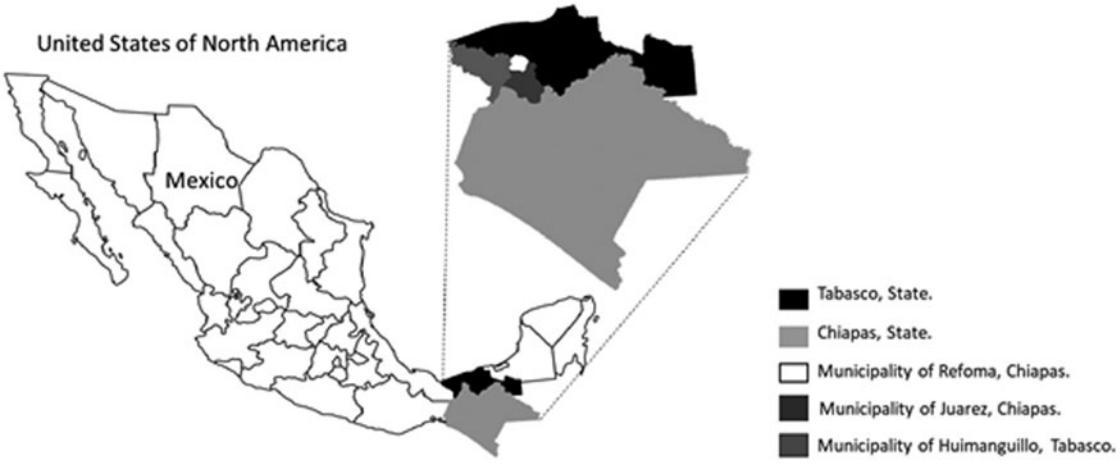

Fig. 1. Localities of origin of the inspected cattle for the collection of rumen fluke eggs.

2016) and Asia (Ali *et al.*, 2018). In America, the presence of rumen flukes in ruminants has been reported in Peru (Pinedo *et al.*, 2010) and Mexico (Ojeda-Robertos *et al.*, 2014).

Despite the widespread infections currently caused by rumen flukes, few studies have examined the efficacy of anthelmintic drugs to control these infections (Fairweather *et al.*, 2012; Huson *et al.*, 2017). On the other hand, a large number of studies have been published on the efficacy of anthelmintic drugs against *F. hepatica* (Fairweather *et al.*, 2012; Robles-Pérez *et al.*, 2014, 2015; Novobilský *et al.*, 2016; Zhang *et al.*, 2019). Anthelmintic drugs should be effective against developing eggs and immature and adult rumen flukes. Therefore, the objective of the present study was to evaluate the efficacy of several commonly used anthelmintic drugs against the egg development of rumen flukes recovered from ruminants raised in the humid tropics of Mexico.

Materials and methods

Study area

Rumen flukes were collected from animals slaughtered in a regional abattoir in the municipality of Juarez, Chiapas, Mexico, located at 17°41'N and 93°13'W. Cattle slaughtered in this abattoir were from the municipality of Huimanguillo in the state of Tabasco and the municipalities of Reforma and Juarez in the state of Chiapas. Figure 1 shows the location of these municipalities. Climatic conditions corresponded with those of tropical humid rainforest, with an average temperature of 26°C and more than 2000 mm of annual rainfall (CONAGUA, 2019).

Collection of rumen flukes and eggs

The rumen and reticulum of the slaughtered cattle were inspected, and the observed flukes were collected. In total, 50 parasites were placed in 50-ml centrifuge tubes containing 10 ml of sterile water. Subsequently, rumen flukes were washed again with sterile water to eliminate cellular detritus. Rumen fluke eggs were obtained placing the parasites in 50-ml centrifuge tubes containing 10 ml of RPMI solutions for five hours at room temperature. Afterwards, a second washing step was carried out with sterile water, and the excreted eggs were screened with a 37-µm sieve. The recovered eggs were transferred to 15-ml tubes with 10 ml of sterile water.

Quantification of rumen fluke eggs

Rumen fluke eggs were counted by adjusting the volume in the centrifuge tubes to 10 ml with sterile water. The tubes were mixed in a vortex to homogenize the eggs throughout the mixture. The egg counts were then performed on a sub-sample of ten aliquots of 10 µl. The average egg count was then extrapolated to the total volume. Subsequently, the volume was diluted to one egg per microliter and streptomycin sulphate (10 mg/l) and benzyl penicillin (9900 units/l) was added at room temperature (27°C) for *in vitro* assays.

Identification of adult parasites

The flukes were identified based on their morphology (Eduardo, 1982), and were subsequently fixed in formalin and dehydrated. Ten specimens of each isolate were measured to discriminate species by size (Nikander & Saari, 2007). Other specimens were hydrated and stained with haematoxylin–eosin and mounted on the slides to differentiate them by their species characteristics.

In vitro assays to evaluate efficacy of flukicidal drugs against egg development

In vitro assays were performed using an established technique to evaluate the development of *F. hepatica* eggs (Fairweather *et al.*, 2012) with several modifications. Briefly, 96-well polystyrene microtiter plates (NUNC Maxisorb™ Invitrogen by Thermo Fisher Scientific, Waltham, Massachusetts, U.S.) were used. A single anthelmintic drug was tested in each row, evaluating a total of 12 dilutions. One hundred eggs were placed into the wells, which were filled with 350 µl of sterile water. The following drugs were tested: rafoxanide at a concentration of 0.0015–3.0 mg/ml (Rafoxcur™ 200 mg/ml; Riverfarma, Mexico City, Mexico), nitroxinil at a concentration of 0.0025–10.20 mg/ml (Nitroxinil 34%; Nitromic™, Microsules, Canelones, Uruguay) and closantel at a concentration of 0.0015–3 mg/ml (Closantel 10%; Closiver ADE™, Andoci, Mexico City, Mexico). The control group was kept under similar conditions without added anthelmintic drugs. Each assay was replicated four times to ensure the veracity of the results.

The microtiter plates were placed in a dark incubator at a room temperature of 26°C and monitored for 14 days. The plates were

https://doi.org/10.1017/S0022149X20000607 Published online by Cambridge University Press

Table 1. Prevalence and coding of paramphistomide strains according to their origin and date of collection.

Species	State	Coding	Date	Number of animals		Prevalence
				Positives	Total	
Calicophoron brothriophoron	Chiapas	JC24519	24/05/2019	8	30	0.27
Calicophoron brothriophoron	Chiapas	JRO7619	07/06/2019	5	15	0.33
Calicophoron clavula	Tabasco	HP7619	07/06/2019	6	14	0.43
Calicophoron clavula	Chiapas	RM28619	28/06/2019	12	24	0.50
Paramphistomum cervi	Chiapas	JRJ19719	19/07/2019	11	36	0.31

exposed to daylight (approximately 12 h) one day before the evaluation to stimulate hatching. The following day, the content of each well was examined under an optic microscope (10×), and the number of eggs in each stage of development was recorded according to Fairweather *et al.* (2012).

The efficacy of flukicidal drugs was evaluated by probit analysis. Then, rumen fluke eggs were classified as non-viable (dead, empty, unembryonated or under cell division without movement or pulsation after 15 days of incubation) or viable (eye spots, hatching or hatched) (Fairweather *et al.*, 2012).

Statistical analysis

The proportion of non-viable and viable eggs was calculated to obtain the median lethal dose (LD_{50}) and maximum lethal dose (LD_{99}) using a logistic regression model (probit) in the SAS program (SAS, 2017):

$$\begin{aligned}\Pr(\text{Response}) &= C + (1 - C)\, F(x'\beta) \\ &= C + (1 - C)\, \Phi(b_0 + b_1 \times \log_{10}(\text{Dose}))\end{aligned}$$

where β = vector of parameter estimates, F = cumulative distribution function (normal, logistic or extreme value), x = vector of explanatory variables, Pr = probability of a response, C = natural (threshold) response rate and Φ = the normal cumulative distribution function.

Results

Rumen flukes from a total of 119 animals were evaluated in the abattoir. Three different species of rumen flukes were taxonomically identified: *Calicophoron brothriophoron* was found in cattle from two localities in Juarez, Chiapas (JC24519 and JRO7619); *Calicophoron clavula* in cattle from Huimanguillo, Tabasco (HP7619), and Reforma, Chiapas (RM28619); and *Paramphistomum cervi* in cattle from Juarez, Chiapas (JRJ19719). The number of animals from each locality, number of infected animals and prevalence of rumen flukes in the inspected animals are shown in table 1.

The lethal doses of the analysed anthelmintic drugs are shown in table 2. Closantel caused the 100% mortality of isolated JRJ19719$^{\Phi}$ at all tested doses. Therefore, no values are reported in the probit analysis. However, closantel resulted in high variability in the LD_{50} values of the different analysed isolates (17–122 µg/ml). In particular, the isolated RM28619 presented high mortality at all evaluated doses (fig. 2).

Rafoxanide showed the lowest efficacy against egg development. The LD_{50} values for all studied isolates were very high, from 500 to 1713 µg/ml (fig. 3). Moreover, the LD_{50} and LD_{99} values for the isolated JRJ19719 were not significant because mortality was similar between them and the control group. Also, it was not effective against eggs of *P. cervi*.

Nitroxinil showed the highest efficacy against egg development. The cases in which the regression slope was not significant occurred due to the high mortality at the lowest doses. The LD_{50} values of nitroxinil were 0.11–65 µg/ml, and the lethal doses in even the non-significant cases corresponded with high mortality (table 2 and fig. 4).

The analysis of each developmental category of eggs (viable/non-viable) showed that 20% of eggs died naturally and above the LD_{50} threshold.

Discussion

The present study is the first to examine the effects of flukicidal drugs against *in vitro* egg development of common rumen flukes in the humid Mexican tropics. The flukicidal effects of anthelmintic drugs in cattle have generally been tested *in vivo* (Rolfe & Boray, 1987). Rumen flukes can cause significant damage to their ruminant hosts, affecting rumen papillae and causing acanthosis and/or hyperkeratosis of the epithelium. Infiltration of inflammatory cells is often related to epithelial changes in the duodenal mucosa and submucosa (Fuertes *et al.*, 2015). Also, metacercariae from cattle can possibly contaminate vegetables for human consumption, resulting in zoonotic infections (González-Warleta *et al.*, 2013; Sanchís *et al.*, 2013; Ferreras *et al.*, 2014; Khedri *et al.*, 2015; Huson *et al.*, 2017; Ploeger *et al.*, 2017).

The inadequate use of anthelmintic drugs has generated an increase in the resistance of diverse species of parasites in recent times. This can result in high economic losses to livestock production systems, especially considering that flukicidal drugs are expensive (Zhang *et al.*, 2019).

An indirect technique to determine the efficacy of flukicidal drugs is through i*n vitro* assays. This technique evaluates the effect of different drugs on the development of fluke eggs, which contain the genetic information on the next generation of flukes. Recently, *in vitro* protocols to evaluate flukicidal drugs against *F. hepatica* eggs were developed (Fairweather *et al.*, 2012; Canevari *et al.*, 2014). Subsequently, the methods to determine the viability and development of *Calicophoron daubneyi* eggs were standardized (Chryssafidis *et al.*, 2015). The methods of the present study were based on these previous studies.

The results showed high variability in the development and response of rumen fluke eggs following exposure to flukicidal

https://doi.org/10.1017/S0022149X20000607 Published online by Cambridge University Press

Table 2. Significance and values of the regression parameters (β_0 and β_1), natural mortality rate (C), mean lethal dose 50 (LD_{50}) and 99 (LD_{99}) in paramphistomide eggs in different strains.

	β0	β_1	C	Intercept	Log (Conc)	LD_{50} (µg/ml)	LD_{99} (µg/ml)
Rafoxanide							
JC24519	**	**	0.219	0.58	2.24	553.0	6028
JRO7619	*	*	0.222	0.61	2.26	538.0	5763
HP7619	ns	**	0.165	−2.06	8.80	1713.0	3149
RM28619	ns	**	0.274	−0.31	4.96	1154.4	3397
JRJ19719[∞]	ns	ns	0.292	−11.39	22.62	(3190.2)	(4040)
Closantel							
JC24519	ns	*	0.163	2.46	1.57	27.5	826
JRO7619	**	**	0.419	8.46	9.25	122.0	217
HP7619	**	**	0.120	2.13	1.21	17.4	1463
RM28619[ϱ]	**	ns	0.254	5.09	1.20	(0.058)	(4.99)
JRJ19719[ϕ]	–	–	–	–	–	–	–
Nitroxinil							
JC24519	**	**	0.175	1.67	0.42	0.1	37310
JRO7619[ϱ]	**	ns	0.107	2.28	0.15	(8.30×10^{-13})	(1880)
HP7619[ϱ]	**	ns	0.107	1.65	0.14	(1.47×10^{-9})	(6.9×10^{7})
RM28619	**	**	0.252	4.43	3.32	46.3	232
JRJ19719	**	**	0.272	4.1244	3.48	65.3	304

[ϕ]100% mortality in all doses; [∞]not effective against eggs; [ϱ]highly effective product. Conc, product concentration; ns, slope was not significant; β_0, intercept; *Significant variable with $p < 0.005$; **Significant variable with $p < 0.01$.

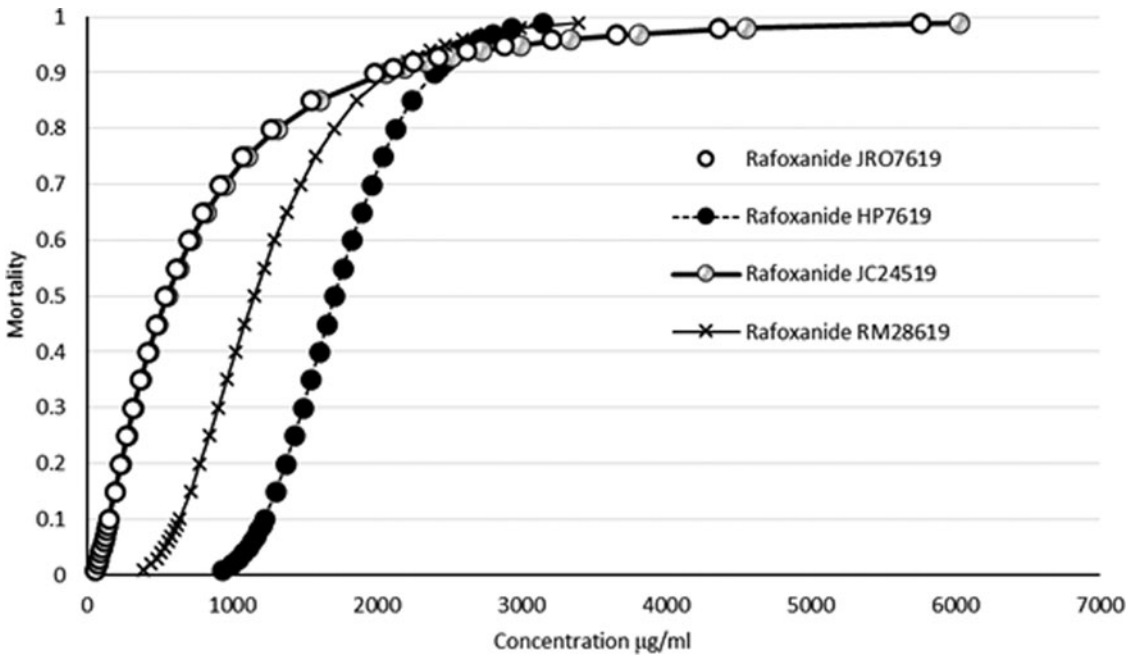

Fig. 2. Relationship of the mortality of eggs of four isolates of paramphistomides with respect to the dose used of rafoxanide.

drugs. This could be associated with the frequent use of the same drugs to control rumen flukes, which might result in greater resistance (González-Garduño *et al.*, 2019). It is also notable that the number of non-viable eggs was high in all treatments, reflecting the naturally high rate of egg mortality in rumen flukes. Similar results were reported by Chryssafidis *et al.* (2015).

In cases where the probit regression slope was not significant (β_1), the LD_{50} and LD_{99} values had no biological

https://doi.org/10.1017/S0022149X20000607 Published online by Cambridge University Press

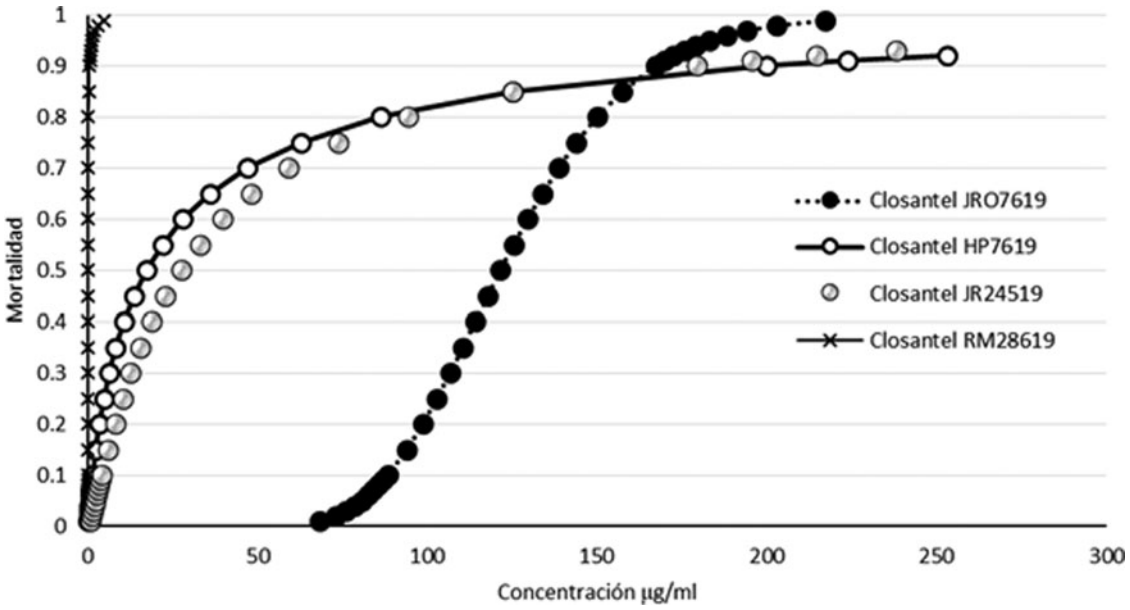

Fig. 3. Relationship of the mortality of eggs of four isolates of paramphistomides with respect to the dose used of Closantel.

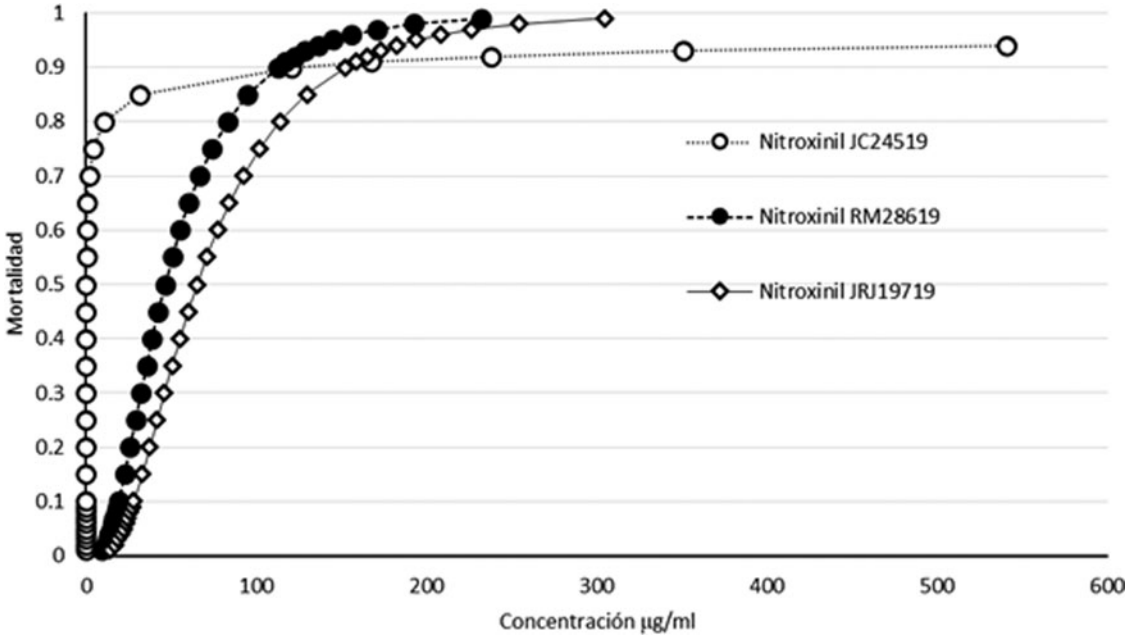

Fig. 4. Relationship of the mortality of eggs of four isolates of paramphistomides with respect to the dose used of nitroxinil.

significance. This implies that there is no dose-dependent effect, and two situations can be occurring: (a) the estimated dose was too high, implying low efficacy, such as occurred with rafoxanide; or (b) the drug was so highly effective that all eggs died at the lowest doses. Therefore, when the mortality was 100% in all wells, the probit analysis could not be performed because no dose-dependent effect was observed, as occurred with closantel. In fact, closantel and nitroxinil presented a good inhibitor effect on egg development that was not observed for rafoxanide.

Rafoxanide and closantel are compounds belong to halogenated salicylanilides, which have similar physicochemical characteristics, such as their molecular weight (rafoxanide 626 and closantel 663 g/mol), their potent inhibitor effect on electron transport associated with mitochondrial phosphorylation and adenosin triphosphate production, are highly lipid-soluble and have low solubility in water (Swan, 1999). The availability of closantel depends on binding to plasma proteins (mainly albumin), and it has been suggested that in the digestive tract; it depends on proteins or carrier molecules to reach the target sites (Rothwell

https://doi.org/10.1017/S0022149X20000607 Published online by Cambridge University Press

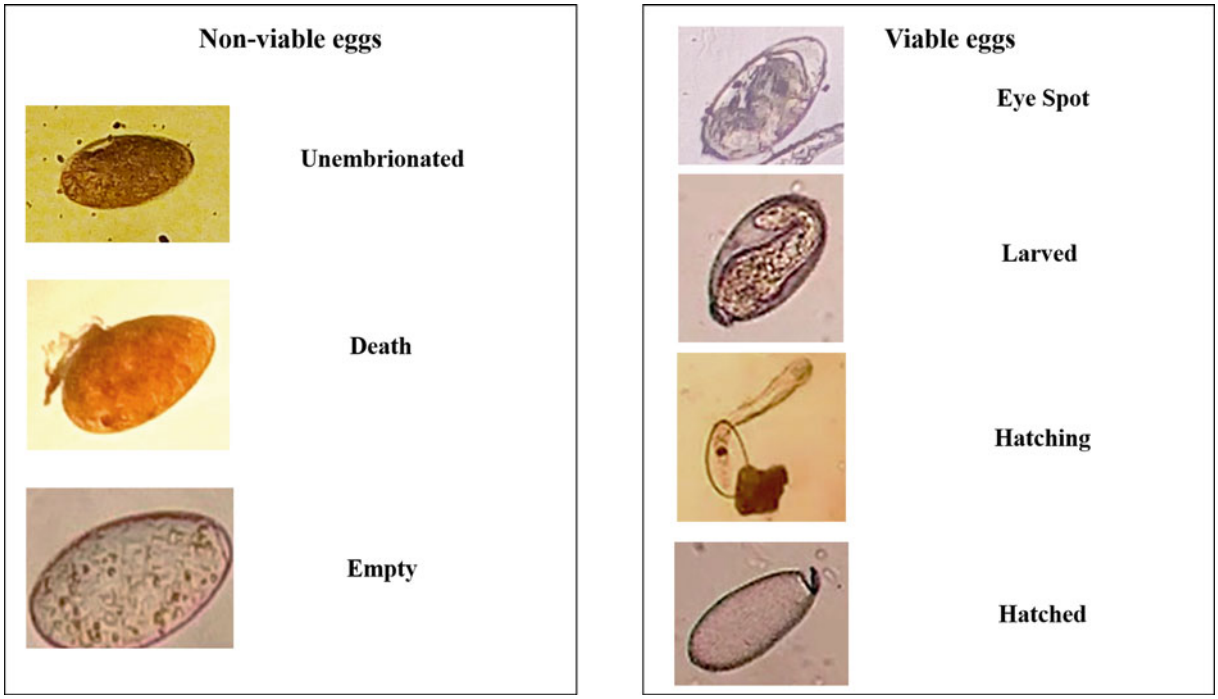

Fig. 5. Classification of stages of development of rumen fluke eggs.

et al., 2000). Nitroxinil is a halogenated phenol, unlike rafoxanide and closantel; this compound has an adequate solubility in water and its molecular weight (290 g/mol) is less than these compounds (Rahman *et al.*, 2017). These nitroxinil characteristics could contribute to passing through the eggshell and affect the viability of the eggs more effectively than rafoxanide and closantel.

The technique to assess anthelmintic resistance based on the classification of seven different categories of eggs previously described by Fairweather *et al.* (2012) was not practical and took a long time. Moreover, they described a category corresponding to the 'cell division stage', which is reached after approximately seven to eight days of incubation (Fairweather *et al.*, 2012). The proportion of eggs in this category was analysed 15 days after incubation yet did not present movement or pulsation and, therefore, did not reach the following development stage. Accordingly, the eggs that presented these characteristics were considered non-viable. To avoid the use of multiple categories in the egg development and thereby facilitate the interpretation of the results, the probit analyses were performed using only the non-viable and viable egg classifications (fig. 5).

Previous *in vivo* evaluations of flukicidal drugs have often been based on faecal count reduction tests. These tests suppose that egg production is due to the ineffectiveness of the drugs utilized to eliminate adult flukes. One study carried out in dairy cattle naturally infected by *C. daubneyi* showed that egg output was not fully suppressed by the studied drugs. A faecal count reduction of 0–26% was found in cows receiving albendazole and netobimin, and 97–99% in cows receiving closantel and oxyclozanide (Arias *et al.*, 2013). In contrast to the present study, a previous study reported the inefficacy of closantel in treating rumen fluke infections in three different herds (Malrait *et al.*, 2015). Meanwhile, a study in sheep reported that oxyclozanide was 99% effective against *Paramphistomum leydeni adults* (Sanabria *et al.*, 2014).

Compared to *in vivo* studies, *in vitro* assays can prevent unnecessary costs through first screening flukicidal drugs and identifying those with little efficacy. It is important to understand which flukicidal drugs affect the viability of eggs and to what extent (Chryssafidis *et al.*, 2015) to control future infections in herds. Interference in the life cycle of flukes can potentially reduce the risk of infection to final hosts. For example, the production of miracidia can also be delayed by flukicidal drugs (Sanabria & Romero, 2008), especially those with a half-life of several days, such as closantel and nitroxinil (Swan, 1999).

Given the re-emergence of rumen fluke infections in several countries of the world, it is important to understand the basic biology of flukes in order to develop diagnostic tools. Also, as shown herein, it is important to test the effectiveness of the different flukicidal drugs available for controlling these infections in order to decrease the negative effects on livestock.

Conclusions

The evaluated flukicidal drugs presented differential efficacy against the development of rumen fluke eggs. Nitroxinil presented the highest efficacy in stopping the development of eggs, whereas rafoxanide presented the lowest. Closantel presented variable efficacy depending on the evaluated isolated.

Financial support. This work was supported by the General Department of Research and Postgraduate Studies of the Universidad Autónoma Chapingo (grant number 20010-C-67).

Conflicts of interest. None.

Ethical standards. The procedures were carried out in accordance with the Official Mexican Standard 051-ZOO-1995 for humanitarian treatment of mobilized animals.

https://doi.org/10.1017/S0022149X20000607 Published online by Cambridge University Press

References

Ali Q, Rashid I, Shabbir MZ, Akbar H, Shahzad K, Ashraf K and Chaudhry U (2018) First genetic evidence for the presence of the rumen fluke *Paramphistomum epiclitum* in Pakistan. *Parasitology International* **67**(5), 533–537.

Arias MSS, Sanchís M, Francisco I, *et al.* (2013) The efficacy of four anthelmintics against *Calicophoron daubneyi* in naturally infected dairy cattle. *Veterinary Parasitology* **197**, 126–129.

Canevari J, Ceballos L, Sanabria R, *et al.* (2014) Testing albendazole resistance in Fasciola hepatica: validation of an egg hatch test with isolates from South America and the United Kingdom. *Journal of Helminthology* **88**, 286–292.

Cauquil L, Hüe T, Hurlin JC, Mitchell G, Searle K, Skuce P and Zadoks R (2016) Prevalence and sequence-based identity of rumen fluke in cattle and deer in New Caledonia. *PLoS ONE* **11**(4), 1–15.

Chryssafidis AL, Fu Y, De Waal T and Mulcahy G (2015) Standardisation of egg-viability assays for *Fasciola hepatica* and *Calicophoron daubneyi*: a tool for evaluating new technologies of parasite control. *Veterinary Parasitology* **210**(1–2), 25–31.

CONAGUA (2019) Servicio Meteorológico Nacional. Normales climatológicas. Resúmenes mensuales de temperatura y lluvia. https://smn.conagua.gob.mx/es/climatologia/temperaturas-y-lluvias/resumenes-mensuales-de-temperaturas-y-lluvias (accesed on March 20, 2019).

Eduardo SL (1982) The taxonomy of the family Paramphistomidae Fischoeder, 1901 with special reference to the morphology of species occurring in ruminants. I. General considerations. *Systematic Parasitology* **4**(1), 7–57.

Fairweather I, McShane DD, Shaw L, Ellison SE, O'Hagan NT, York EA, Trudgett A and Brennan GP (2012) Development of an egg hatch assay for the diagnosis of triclabendazole resistance in *Fasciola hepatica*: proof of concept. *Veterinary Parasitology* **183**(3–4), 249–259.

Ferreras MC, González-lanza C, Pérez V, *et al.* (2014) *Calicophoron daubneyi* (Paramphistomidae) in slaughtered cattle in Castilla y León (Spain). *Veterinary Parasitology* **199**(3–4), 268–271.

Fuertes M, Pérez V, Benavides J, González-Lanza MC, Mezo M, González-Warleta M and Ferreras MC (2015) Pathological changes in cattle naturally infected by *Calicophoron daubneyi* adult flukes. *Veterinary Parasitology* **209**(3–4), 188–196.

González-Garduño R, Hernández-Hernández JC, Ortiz-Pérez DO and Torres-Hernández G (2019) Hematological performance of cattle infected by trematodes in a humid warm climate of Mexico. *Pastos y Forrajes* **42**(3), 185–188.

González-Warleta M, Lladosa S, Castro-Hermida JA, Martínez-Ibeas AM, Conesa D, Muñoz F, López-Quílez A, Manga-González Y and Mezo M (2013) Bovine paramphistomosis in Galicia (Spain): prevalence, intensity, aetiology and geospatial distribution of the infection. *Veterinary Parasitology* **191**(3–4), 252–263.

Huson KM, Oliver NAM and Robinson MW (2017) Paramphistomosis of ruminants: an emerging parasitic disease in Europe. *Trends in Parasitology* **33**(11), 836–844.

Jones RA, Brophy PM, Mitchell ES and Williams HW (2017) Rumen fluke (*Calicophoron daubneyi*) on Welsh farms: prevalence, risk factors and observations on co-infection with *Fasciola hepatica*. *Parasitology* **144**(2), 237–247.

Khedri J, Radfar MH, Borji H and Mirzaei M (2015) Prevalence and intensity of *Paramphistomum* spp. in cattle from south-eastern Iran. *Iranian Journal of Parasitology* **10**(2), 268–272.

Malrait K, Verschave S, Skuce P, Van Loo H, Vercruysse J and Charlier J (2015) Novel insights into the pathogenic importance, diagnosis and treatment of the rumen fluke (*Calicophoron daubneyi*) in cattle. *Veterinary Parasitology* **207**(1–2), 134–139.

Millar M, Colloff A and Scholes S (2012) Disease associated with immature paramphistome infection. *Veterinary Record* **171**, 509–510.

Nikander S and Saari S (2007) Notable seasonal variation observed in the morphology of the reindeer rumen fluke (*Paramphistomum leydeni*) in Finland. *Rangifer* **27**(1), 47–57.

Novobilský A, Amaya Solis N, Skarin M and Höglund J (2016) Assessment of flukicide efficacy against *Fasciola hepatica* in sheep in Sweden in the absence of a standardised test. *International Journal for Parasitology: Drugs and Drug Resistance* **6**(3), 141–147.

Ojeda-Robertos NF, Medina-Reynes A, Garduza-Arias G and Rangel-Ruiz LJ (2014) Dinámica de excreción de huevos de Fasciola hepatica y Paramphistomum spp en ganado bovino de Tabasco. *Ecosistemas y Recursos Agropecuarios* **1**(1), 73–80.

Pavan Kumar C, Syaama Sundar N and Devi Prasad V (2016) Outbreak of immature paramphistomosis in nellore jodipi sheep. *Journal of Parasitic Disease* **40**, 533–535.

Pinedo V, Chávez V, Casas A, Suárez A, Sánchez P and Huamán U (2010) Prevalencia de trematodes de la familia Paramphistomatidae en bovinos del distrito de Yurimaguas, provincia de alto Amazonas. *Revista de Investigaciones Veterinarias Del Perú* **21**(2), 161–167.

Ploeger HW, Ankum L, Moll L and Holzhauer M (2017) Presence and species identity of rumen flukes in cattle and sheep in the Netherlands. *Veterinary Parasitology* **243**, 42–46.

Rahman M, Kabir A, Ahmed S, *et al.* (2017) Nitroxynil uncouples oxidative phosphorylation in the cell mitochondria and a drug wherever injectables are preferred over drenching. *Bangladesh Journal of Veterinary Medicine* **15**, 922–2308.

Robles-Pérez D, Martínez-Pérez JM, Rojo-Vázquez FA and Martínez-Valladares M (2014) Development of an egg hatch assay for the detection of anthelmintic resistance to albendazole in *Fasciola hepatica* isolated from sheep. *Veterinary Parasitology* **203**(1), 217–221.

Robles-Pérez D, Martínez-Pérez JM, Rojo-Vázquez FA and Martínez-Valladares M (2015) Screening anthelmintic resistance to triclabendazole in *Fasciola hepatica* isolated from sheep by means of an egg hatch assay. *BMC Veterinary Research* **11**, 226.

Rojo-Vázquez F, Meana A, Valcárcel F and Martínez-Valladares M (2012) Update on trematode infections in sheep. *Veterinary Parasitology* **189**(1), 15–38.

Rolfe PF and Boray JC (1987) Chemotherapy of paramphistomosis in cattle. *Australian Veterinary Journal* **64**(11), 328–332.

Rothwell JT, Lacey E and Sangster NC (2000) The binding of closantel to ovine serum albumin, and homogenate fractions of *Haemonchus contortus*. *International Journal for Parasitology* **30**, 769–775.

Sanabria REF and Romero JR (2008) Review and update of paramphistomosis. *Helminthologia* **45**(2), 64–68.

Sanabria R, Moreno L, Alvarez L, Lanusse C and Romero J (2014) Efficacy of oxyclozanide against adult *Paramphistomum leydeni* in naturally infected sheep. *Veterinary Parasitology* **206**(3–4), 277–281.

Sanchís J, Sánchez-Andrade R, Macchi MI, Piñeiro P, Suárez JL, Cazapal-Monteiro C and Arias MS (2013) Infection by Paramphistomidae trematodes in cattle from two agricultural regions in NW Uruguay and NW Spain. *Veterinary Parasitology* **191**(1–2), 165–171.

Sargison N, Francis E, Davison C, Bronsvoort BMdC, Handel I and Mazeri S (2016) Observations on the biology, epidemiology and economic relevance of rumen flukes (Paramphistomidae) in cattle kept in a temperate environment. *Veterinary Parasitology* **219**, 7–16.

SAS (2017) SAS/STAT user's guide (S. Inst., Ed.), release 6, Cary, NC, USA.

Swan GE (1999) The pharmacology of halogenated salicylanilides and their anthelmintic use in animals: review article. *Journal of the South African Veterinary Association* **70**(2), 61–70.

Tehrani A, Javanbakht J, Khani F, Hassan MA, Khadivar F, Dadashi F, Alimohammadi S and Amani A (2015) Prevalence and pathological study of Paramphistomum infection in the small intestine of slaughtered ovine. *Journal of Parasitic Diseases* **39**(1), 100–106.

Toolan DP, Mitchell G, Searle K, Sheehan M, Skuce PJ and Zadoks RN (2015) Bovine and ovine rumen fluke in Ireland-Prevalence, risk factors and species identity based on passive veterinary surveillance and abattoir findings. *Veterinary Parasitology* **212**(3–4), 168–174.

Zhang J-L, Si H-F, Zhou X-Z, Shang X-F, Li B and Zhang JY (2019) High prevalence of fasciolosis and evaluation of the efficacy of anthelmintics against *Fasciola hepatica* in buffaloes in Guangxi, China. *International Journal for Parasitology. Parasites and Wildlife* **8**, 82–87.

https://doi.org/10.1017/S0022149X20000607 Published online by Cambridge University Press