García-Dios *et al. BMC Veterinary Research* (2025) 21:561
https://doi.org/10.1186/s12917-025-05009-y

BMC Veterinary Research

RESEARCH Open Access

Beyond stabilization: prevalence, risk factors and molecular identification of rumen flukes in cattle from Northwestern Spain

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Abstract

Background An upward trend of paramphistomid prevalence was detected in domestic ruminants from Europe in the last decades. Nevertheless, recent data from Ireland, which reports the highest prevalences in Europe, suggests that this trend may be stabilizing. This study analysed the current epidemiological situation of rumen fluke infections in cattle from northwestern Spain, focusing on two regions: Galicia, where increasing prevalences were reported, and Asturias, where data is limited. Between 2018 and 2022, 3,095 faecal samples from 137 farms were analysed using sedimentation coproscopical technique. Risk factor analysis was conducted through mixed logistic regression and ANOVA; paramphistomid species were molecularly identified.

Results High individual (51.2%; 95% CI: 49.4–53.0) and herd (81.8%; 95% CI: 74.1–87.6) prevalences were found, representing the highest recorded prevalence of paramphistomid infections in cattle from Spain. The risk of being infected significantly increased with age, being lower in cattle aged 1–24 months than in those 25–60 months-old (OR 5.8) or older than 60 months (OR 17.1). Prevalence was significantly higher in Asturias (OR 11.4) and in animals co-infected with *Fasciola hepatica* (OR 13.1). Animals from farms without slurry scrapers also showed significantly higher prevalences (OR 76.9), which may be related to less professionalization. Egg shedding intensity was notably higher in older animals and those co-infected with *F. hepatica* as well as in farms from coastal areas and using extensive management. *Calicophoron daubneyi* was the only species detected.

Conclusions Our findings reveal a continued upward trend in rumen fluke prevalence in cattle from north-western Spain, suggesting that a stabilization of prevalence is not occurring. The study highlights that surveillance, farmer education, and integrated control measures are highly recommended in north-western Spain, especially in Asturias, where infection rates are particularly high.

Keywords Paramphistomids, *Calicophoron daubneyi*, Cattle, Epidemiology, Risk factors, PCR, Spain

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Background

Cattle sector has a significant economic importance in north-western Spain. Over recent decades, this sector has experienced considerable changes. The traditional family farms, which once predominated in this region, have been replaced by larger, fewer, and significantly more professionalized farms [1, 2]. In addition, in recent years, the growing concern on animal welfare and sustainable production practices has prompted changes in management systems, such as the return to grazing practices. These changes in management have influenced the assessment of on-farm parasitic risks, thereby raising concerns regarding gastrointestinal nematode or trematode infections, including those caused by paramphistomids.

Paramphistomids, also known as rumen flukes, are digenean trematodes whose adults are located in pre-stomachs of their definitive hosts, including domestic and wild ruminants [3]. These parasites require an intermediate host, a freshwater snail [4–8]. In cattle, clinical signs are more frequent in young animals after a massive ingestion of metacercariae within a short time [9], leading to significant duodenal lesions and considerable economic losses [10–12]. Although these trematodes are prevalent in tropical and subtropical areas, their occurrence in Europe was considered unusual until the last decades of the 20th century. Nevertheless, during the 1990s, a significant rise in the prevalence was documented in France [13]. Since then, the spread of paramphistomids has been observed across nearly all the European continent [3], with the highest prevalences recorded in cattle from western Europe [14, 15]. This increase in the prevalence rates has been mainly related to the spread of *Calicophoron daubneyi* across the European continent. This paramphistomid species has adapted effectively to *Galba truncatula*, an amphibious snail that is very abundant in Europe, also acting as an intermediate host of the liver fluke *Fasciola hepatica* [6].

In Spain, the presence of paramphistomids has been exhaustively studied in ruminants from the north-western area of the country, especially in cattle from Galicia, which is considered an endemic area for fasciolosis where *G. truncatula* is abundant [16]. In this region, a number of investigations reported low but stable prevalences of paramphistomids (12–17%) during the early 2000s [17–19]; nevertheless, a progressive increase in the prevalence of paramphistomids in cattle (18.8–26%) has been observed from 2010 onwards [11, 20]. The most recent data on rumen fluke infections in cattle from northern Spain indicates an overall prevalence of 33.9% in organic farms from four regions: Galicia, Asturias, Cantabria, and the Basque Country [21]. Since then, no further epidemiological investigations on paramphistomid infection in cattle were performed in this area. It is worth noting that in countries with high prevalences, such as Ireland or France, recent data show a maintenance or reduction of infection rates suggesting a possible stabilization of the situation [13, 14, 22, 23]. Thus, obtaining updated data on the presence of these trematodes in north-western Spain is strongly needed.

In this context, the present study provides updated information on the prevalence of paramphistomids in cattle from two regions in north-western Spain. Thus, the findings from Galicia -where information on rumen fluke infections in cattle is available for more than 25 years and training of farmers on this issue was extensively performed- and from Asturias -where individualised data is not available and the parasite is less known among farmers- were compared. In addition, risk factors influencing both the probability of infection and the egg shedding were detected, and the paramphistomid species present were identified using molecular methods.

Methods

Study area

The present study was conducted in Galicia (41º 49’ to 43º 47’ N, 6º 42’ to 9º 18’W) and Asturias (42°54’ to 43°39’ N, 4°25’ to 7°11’ W), two regions located in north-western Spain and characterized by its oceanic climate, with high rainfall and moderate temperatures throughout the year [24, 25]. The study area has a population of 1,297,012 heads of cattle, representing 20.3% of the cattle population in Spain; it is worth noting that both regions are key dairy cattle areas, including more than half of the animals destined for milk production in the country (50.2%; 393,045 animals) [26].

Sampling and data collection

The number of farms required for the study was calculated using the n.for.survey function from the EpiDisplay R statistical package [27] considering a 95% confidence interval, a precision of 0.1, and a prevalence of 50%, leading to the highest sampling. Although a minimum of 96 farms was required, additional 41 farms were included for maximizing the territorial coverage; thus, farms were located on 68 different municipalities (Fig. 1). The number of samples collected on each farm was also calculated considering a 95% confidence interval, a precision of 0.1 and a prevalence of 50%. In total, faecal samples from 3,095 cattle were collected between October 2018 and February 2022; animals were only sampled once. The sampling effort was distributed as follows: 6 farms were sampled in 2018 and 2022, while between 36 and 55 farms per year were sampled in the remaining years. All samples were collected directly from the rectum of the animals, stored at 4 °C and analysed individually within 24 h. In each farm, information regarding the age of the animals was sourced from official records, while data on

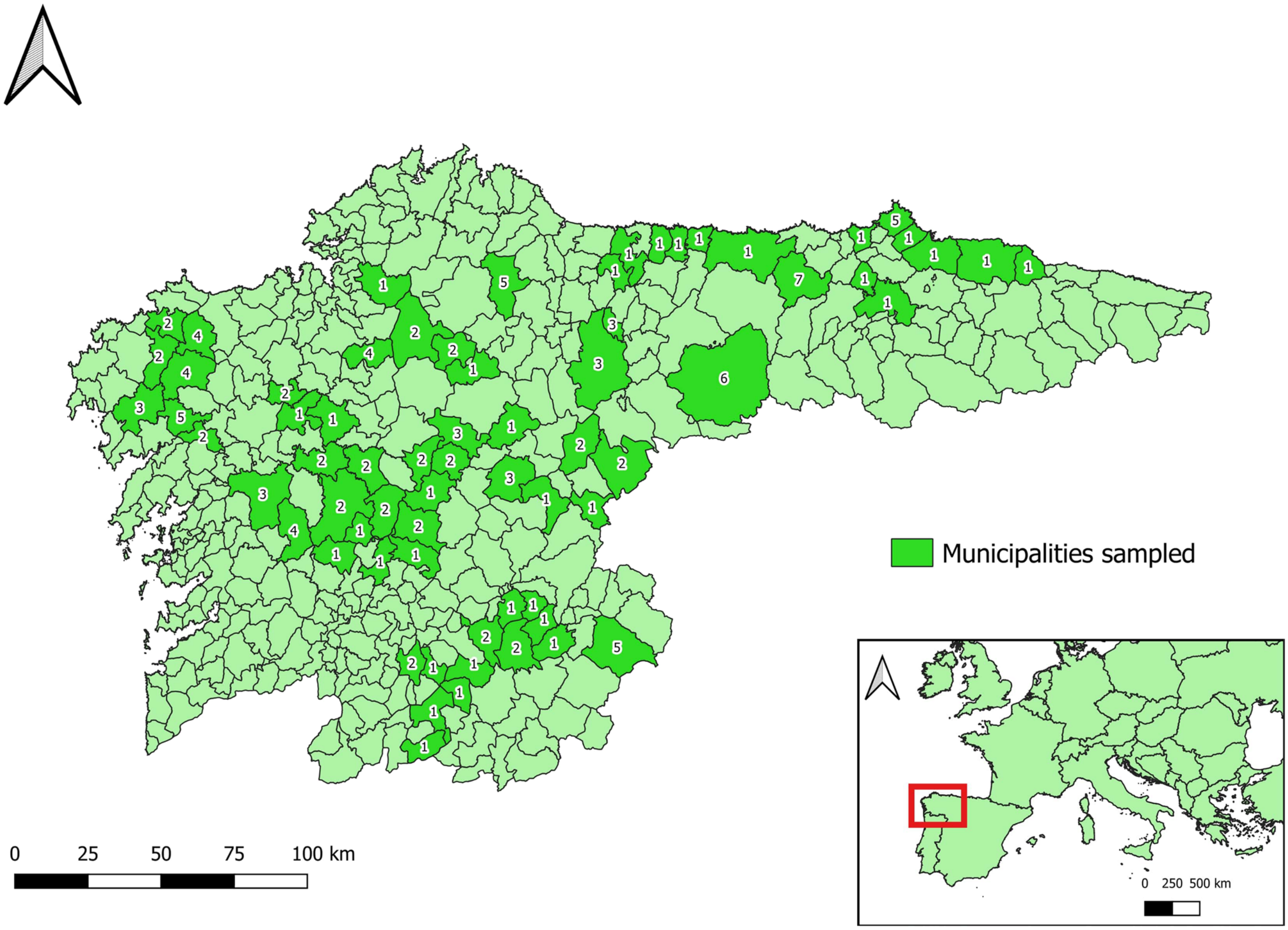

Fig. 1 Location of the cattle farms sampled. The numbers indicate how many farms were sampled in each municipality. Modified from Instituto Geográfico Nacional (IGN) CC-BY 4.0. Instituto Geográfico Nacional (IGN). 2024. Límites municipales, provinciales y autonómicos. Centro Nacional de Información Geográfica (CNIG). [https://centrodedescargas.cnig.es/CentroDescargas/detalleArchivo?sec=9000029#]

management and facilities was gathered using an epidemiological survey.

Coproscopic analysis

Trematode eggs were detected using a quantitative sedimentation technique [28] with some modifications. Thus, 5 g of faeces were processed, the final sediment was diluted into 10 ml of water, and the total volume of two McMaster chambers (2 ml) was examined, allowing achieving a limit of detection of 1 egg per gram of faeces (epg). In addition, a quantitative McMaster flotation technique using saturated saline solution [29] was performed to detect infections by coccidia, gastrointestinal nematodes or cestodes, with a detection limit of 50 eggs/oocysts per gram of faeces (epg/opg).

Risk factor analysis

A mixed logistic regression was performed for identifying factors influencing the probability of paramphistomid infection in cattle within the studied regions; the farm was included as a random variable. This analysis was performed using the glmer() function of the lme4 package [30] in the R statistical package [31]. Factors were manually removed using a stepwise approach (forward and backwards) based on the Akaike information criterion (AIC) and the Bayesian information criterion (BIC) values until the best model was built. Odds ratios were calculated by raising the constant e to the estimators obtained from the model.

The possible influence of different variables on paramphistomid egg shedding was assessed using a multivariate ANOVA; only positive animals ($n = 1586$) were included, and the logarithm of paramphistomid egg counts was used as the dependent variable. To avoid the potential impact of a larger number of animals on some farms, a mixed linear regression was performed -lmer() function in lme4 package-. The factors were similarly eliminated stepwise, forward and backwards, based on the AIC and BIC values until the best model was obtained. Results of the ANOVA over the linear model were obtained using the Anova() function of the car package [32]. Pairwise analyses were performed using the glht() function of the multicomp package [33], using Tukey's adjustment method.

Molecular analysis

The sediment of all the positive samples of each positive farm was pooled; thus, a pool for each positive farm was obtained. DNA was extracted from those pools showing the highest mean egg counts ($n = 41$) using a commercial kit (QIAamp Fast DNA Stool Mini Kit, Qiagen N.V.®, Venlo, The Netherlands) following the manufacturer's instructions. DNA samples were stored at −20 °C until analysis. The samples were tested using a PCR protocol for amplification of a partial region of the internal transcribed spacer of Trematoda described by García-Dios et al. [34]. All PCR-positive samples, including the positive control, were purified and sequenced in both senses on an ABI 3730xl (Applied Biosystems, Foster City, California, USA) using a Big Dye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, California, USA) at the Sequencing and Fragment Analysis Unit of the Santiago de Compostela University (Spain). The sequences were aligned and edited using the software ChromasPro (Technelysium, Brisbane, Australia), and the consensus sequences were queried against the GenBank database using the Basic Local Alignment Search Tool (BLAST; http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Results

Overall prevalence and egg shedding

Overall, 51.2% (95%CI 49.4–53) of the animals shed paramphistomid eggs, showing a mean egg count of 96.6 epg (SD 178.2; range 1–2,744). Half of the positive animals presented counts below 50 epg; eliminations above 250 epg were considered atypical, although some animals exceeded 1,000 epg. At least one infected animal was found in 81.8% (95%CI 74.1–87.6) of the farms. On positive farms, the mean intra-farm prevalence was 65.5% (95%CI 62.6–66.4), although it varied significantly, from 2.4 to 100% (Fig. 2).

Risk factor analysis

A total of 34 independent variables were included in the risk analysis (Table 1). Considering the different factors, the prevalence ranged from 0 to 92.7% and the mean egg shedding of positive animals from 3.8 epg to 244 epg.

The mixed logistic regression model extracted four factors influencing the probability of infection by paramphistomids: region, age, positivity to *F. hepatica* and use of slurry scraper (Table 2).

Statistical analysis revealed that animals from Asturian farms are 11.4 times more likely to be infected with paramphistomids than those from Galicia. Prevalence increased with age; the probability of being positive in animals aged 0-24 months was 5.8 times lower than those aged 25 to 60 months and 17.1 times lower than cattle

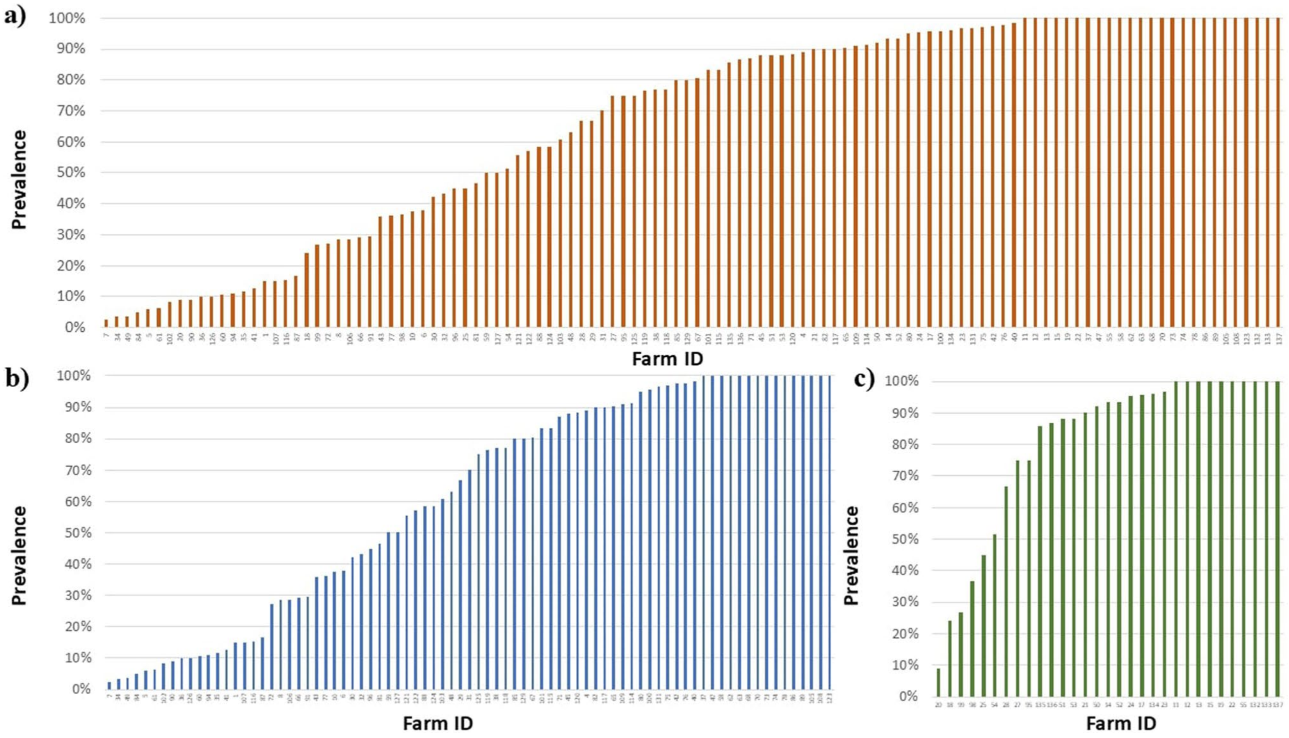

Fig. 2 Intra-herd prevalence of paramphistomid infections within positive farms in total (**a**) and by region (**b**: Galicia; **c**: Asturias)

Table 1 Variables and categories considered in the risk factor analysis for the prevalence and egg shedding of paramphistomids in cattle from Northwestern Spain

Variable	Categories	Positive animals/total (%; CI 95%*)	Arithmetic mean of the egg-count positive animals (SD$^¥$)
Region	Asturias	516/751 (68.7; 65.2–72)	107.3 (209.568)
	Galicia	1,069/2,344 (45.6; 43.6–47.6)	91.4 (160.697)
Climatic area	Plateau	481/1,406 (34.2; 31.7–36.8)	74 (150.260)
	Coast	331/645 (51.3; 47.4–55.2)	123.7 (216.467)
	Mountain	773/1,044 (74; 71.2–76.7)	99.1 (174.437)
Management	Extensive	421/476 (88.5; 85.1–91.1)	136.7 (217.597)
	Semiextensive	1,070/2,104 (50.9; 48.7–53)	85.4 (164.715)
	Intensive	94/515 (18.3; 15.1–21.9)	44.3 (59.420)
Mean altitude	< 550 m	700/1,538 (45.5; 43–48)	116.7 (207.322)
	> 550 m	885/1,557 (56.8; 54.3–59.3)	80.7 (149.412)
Age (months)	1–24	89/251 (35.5; 29.6–41.8)	91.7 (150.613)
	25–60	572/1,325 (43.2; 40.5–45.9)	85.5 (135.862)
	> 60	892/1,425 (62.6; 60-65.1)	185.2 (204.907)
Breed group	Allochthonous	595/1,627 (36.6; 34.2–39)	92.3 (153.829)
	Autochthonous	258/352 (73.3; 68.3–77.8)	112.9 (199.492)
	Unknown	516/816 (63.2; 59.8–66.5)	84.9 (195.687)
	Mixed	216/300 (72; 66.5–76.9)	117 (167.626)
Farm purpose	Meat	909/1,267 (71.7; 69.2–74.2)	93.3 (169.650)
	Dairy	676/1,828 (36.8; 34.6–39)	101 (189.072)
Introduction of animals from other farms	No	755/1,715 (44; 41.7–46.4)	99.7 (153.173)
	Yes	830/1,380 (60.1; 57.5–62.7)	93.8 (198.249)
Presence of other ruminants on the farm	No	1,533/3,024 (50.7; 48.9–52.5)	95.8 (176.301)
	Yes	52/71 (73.2; 61.2–82.7)	121.5 (227.308)
Presence of carnivores on the farm	No	1,011/1,668 (60.6; 58.2–63)	96.1 (174.772)
	Yes	574/1,427 (40.2; 37.7–42.8)	97.5 (184.167)
Proximity to other farms (< 2 km)	No	60/103 (58.3; 48-67.8)	118.8 (133.528)
	Yes	1,525/2,992 (51; 49.2–52.8)	95.7 (179.682)
Quarantine	No	1,363/2,641 (51.6; 49.7–53.5)	99.8 (179.388)
	Yes	222/454 (48.9; 44.2–53.6)	77.3 (169.646)
Use of anthelmintics	No	267/536 (49.8; 45.5–54.1)	66.4 (90.794)
	Yes	1,318/2,559 (51.5; 49.5–53.5)	102.7 (190.512)
Deworming frequency	No	268/537 (49.9; 45.6–54.2)	66.3 (90.652)
	Once a year	443/1,168 (37.9; 35.1–40.8)	98.6 (199.775)
	Twice a year	852/1,318 (64.6; 62-67.2)	105.6 (187.334)
	Three times a year	22/72 (30.6; 20.5–42.7)	78.9 (111.845)
Anthelmintic drugs used	No treatment	268/537 (49.9; 45.6–54.2)	66.4 (90.794)
	Benzimidazole (B)	30/181 (16.6; 11.6–23)	114.8 (166.681)
	Macrocyclic lactones (ML)	689/1,382 (49.9; 47.2–52.5)	104.4 (149.848)
	B + ML	296/458 (64.6; 60–69)	106.3 (203.313)
	Closantel + ML	206/324 (63.6; 58.1–58.8)	122 (298.563)
	ML + Oxyclozanide	81/162 (50; 42.4–57.6)	39.4 (81.737)
Bedding material	Extensive management	411/491 (83.7; 80.1–86.8)	126.1 (197.381)
	Lime	63/92 (68.5; 57.8–77.5)	56.8 (85.853)
	Mat	469/1,018 (46.1; 43-49.2)	88.4 (155.893)
	Straw	168/312 (53.9; 48.1–59.5)	138.9 (287.219)
	Sawdust	105/540 (19.4; 16.2–23.1)	78.7 (139.017)
	Directly on the floor	369/642 (57.5; 53.5–61.3)	66.9 (122.753)
Bed cleaning frequency	Extensive management	423/506 (83.6; 80-86.7)	135.4 (196.160)
	1–2 days	961/2,264 (42.4; 40.4–44.5)	76.2 (153.395)
	4–7 days	75/151 (49.7; 41.5–57.9)	130.6 (218.259)
	14 days or more	126/174 (72.4; 65-78.8)	135.6 (235.001)

Table 1 (continued)

Variable	Categories	Positive animals/total (%; CI 95%*)	Arithmetic mean of the egg-count positive animals (SD$^{¥}$)
Water troughs cleaning frequency	No	84/166 (50.6; 42.8–58.4)	86 (128.405)
	According to use	810/1,396 (58; 55.4–60.2)	98.8 (171.434)
	Daily	272/506 (53.8; 49.3–58.2)	112.7 (226.310)
	2–4 days	162/477 (34; 29.8–38.4)	32.9 (45.002)
	7–14 days	215/469 (45.8; 41.3–50.5)	127.3 (213.249)
	15 days or more	9/44 (20.5; 10.3–35.8)	3.8 (3.308)
	Not applicable	33/37 (89.2; 73.6–96.5)	74.6 (72.034)
Farm floor	Extensive management	405/456 (88.8; 85.5–91.5)	127.9 (198.291)
	Cement	332/392 (84.7; 80.7–88)	62.8 (146.095)
	Straw	57/77 (71.4; 59.8–80.9)	234.9 (377.745)
	Slatted floor	791/1,570 (50.4; 47.9–52.9)	84.8 (148.277)
Corridors cleaning frequency	According to use	41/71 (57.8; 45.5–69.2)	218.5 (248.033)
	Daily	957/2,268 (42.2; 40.2–44.3)	80.6 (163.885)
	3–7 days	105/175 (60; 52.3–67.2)	65.5 (93.389)
	15 days or more	96/146 (65.8; 57.4–73.3)	155.4 (258.252)
	Not applicable	386/435 (88.7; 85.3–91.5)	117.2 (186.937)
Use of slurry scraper	No	1,467/2,355 (62.3; 60.3–64.3)	100.6 (183.168)
	Yes	118/740 (15.9; 13.4–18.8)	46.8 (81.873)
Slurry scraper frequency	No	1,467/2,355 (62.3; 60.3–64.3)	100.6 (183.168)
	1–2 times/day	94/380 (24.7; 20.5–29.5)	49.5 (88.790)
	3 or more times/day	24/360 (6.7; 4.4–9.9)	36.1 (45.530)
Contact with fresh pasture	Never	0/49 (0; 0-0.9)	-
	Occasionally	70/432 (16.2; 12.9–20.1)	37.9 (53.671)
	Daily	1,515/2,614 (58; 56-59.9)	99.3 (181.428)
Pasture rotation	No	128/375 (34.1; 29.4–39.2)	85.6 (153.739)
	Yes	1,433/2,637 (54.3; 52.4–56.3)	98.2 (181.397)
	Not applicable	24/83 (28.9; 19.7–40.1)	63 (71.735)
Presence of water courses on the pasture	No	217/515 (42.1; 37.9–46.5)	124.8 (247.157)
	Yes	1,358/2,511 (54.1; 52.1–56)	92.8 (164.716)
	Not applicable	10/69 (14.5; 7.5–25.5)	6.5 (6.399)
Presence of water troughs on the pasture	No	393/630 (62.4; 58.5–66.2)	63.1 (93.601)
	Yes	1,168/2,382 (49; 47-51.1)	108.6 (198.750)
	Not applicable	24/83 (28.9; 19.7–40.1)	63 (71.735)
Use of slurry as fertilizer	No	11/55 (20; 10.9–33.4)	229.5 (205.874)
	Yes	1,574/3,040 (51.8; 50-53.6)	95.7 (177.692)
Slurry origin	External	7/49 (14.3; 6.4–27.6)	244 (213.115)
	Own	1,578/3,046 (51.8; 50-53.6)	96 (177.813)
Introduction of young animals on the pasture	No	390/875 (44.6; 41.3–47.9)	92.6 (193.424)
	Yes	1,195/2,220 (53.8; 51.7–55.9)	97.9 (172.973)
Age at start of grazing	Not applicable	28/118 (23.7; 16.6–32.6)	61.8 (68.436)
	0 days	489/647 (75.6; 72-78.8)	117.4 (189.725)
	1–30 days	115/175 (65.7; 58.1–72.6)	49.9 (102.202)
	2–5 months	172/293 (58.7; 52.8–64.4)	69.5 (178.410)
	6–9 months	194/552 (35.1; 31.2–39.3)	128.5 (200.929)
	10–14 months	348–620 (56.1; 52.1–60)	97.3 (198.264)
	15–19 months	98/333 (29.4; 24.7–34.7)	71.9 (146.383)
	24 months or more	141/357 (39.5; 34.4–44.8)	73.9 (103.528)
Use of individual boxes for calves	No	1,265/2,143 (59; 56.9–61.1)	91.9 (165.431)
	Yes	320/952 (33.6; 30.6–36.7)	115.1 (220.834)
Positivity to gastrointestinal nematodes	No	1,149/2,350 (48.9; 46.9–51)	95.6 (187.137)
	Yes	435/744 (58.7; 55.1–62.3)	99.6 (152.911)

Table 1 (continued)

Variable	Categories	Positive animals/total (%; CI 95%[*])	Arithmetic mean of the egg-count positive animals (SD[¥])
Positivity to *Fasciola hepatica*	No	1,483/2,985 (49.7; 47.9–51.5)	94.2 (179.586)
	Yes	102/110 (92.7; 85.7–96.6)	130.9 (152.911)
Positivity to *Dicrocoelium dendriticum*	No	1,535/3,034 (50.6; 48.8–52.4)	94.3 (177.731)
	Yes	11/61 (18; 9.8–30.4)	167.7 (178.907)

[*] CI 95%: 95% Confidence Interval

[¥] SD: standard deviation

Table 2 Model obtained by mixed logistic regression for the prevalence of paramphistomids in cattle from Northwestern Spain

Factor	Estimator	Z-value	*p*-value	OR	CI 95%
Region (Galicia)	-	-	-	-	-
Region (Asturias)	2.436	3.234	0.001	11.4	2.6–50
Age 1 (1–24 months)	-	-	-	-	-
Age 2 (25–60 months)	1.765	6.056	< 0.001	5.8	3.3–10.3
Age 3 (> 60 months)	2.840	9.697	< 0.001	17.1	9.64–30.37
Negative to *F. hepatica*	-	-	-	-	-
Positive to *F. hepatica*	2.574	3.711	< 0.001	13.1	3.4–51.1
Use of slurry scraper	-	-	-	-	-
No slurry scraper	4.343	4.609	< 0.001	76.9	12.1-487.8

- Reference category

over 60 months. Significant differences were also found between animals aged 25-60 months and those over 60 months, with the older age group presenting a 2.97-fold higher probability of infection. In addition, infection with F. hepatica significantly influenced the occurrence of paramphistomid infections; thus, animals shedding liver fluke eggs showed a 13.1 times higher probability of having paramphistomid infections. Finally, the probability of infection was up to 76.9 times higher in farms that did not use scrapers.

ANOVA analysis demonstrated that age, climatic area, type of management, and positivity to *F. hepatica* significantly influence egg shedding (Fig. 3). The analysis revealed a clear trend in age: egg counts increased progressively and significantly with age; thus, animals older than 60 months showed the highest egg shedding. Pairwise analyses detected significant differences among all age groups: between animals aged 1–24 months and 25–60 months ($p = 0.003$), between those aged 1–24 months and > 60 months groups ($p < 0.001$), and between cattle aged 25–60 months and > 60 months ($p < 0.001$). In addition, animals from coastal areas showed significantly higher egg shedding than animals from the central area ($p = 0.002$). Regarding management, animals from extensive farms showed significant higher eliminations than those from farms with semiextensive ($p < 0.001$) or intensive ($p = 0.002$) management. Finally, animals positive to *F. hepatica* significantly shed more eggs of paramphistomids than those negative to the liver fluke ($p = 0.006$).

Amplicons of the expected size were obtained in 31 out of the 41 pools analysed. *Calicophoron daubneyi* was the only paramphistomid species identified in all samples. All sequences were identical between them and with those previously obtained in domestic ruminants from different European countries [35–37] including sequences recently obtained in sheep from Galicia [34].

Discussion

Our findings reveal that paramphistomid infections in cattle from north-western Spain keep increasing in recent years, suggesting that infection rates are not stabilizing. The prevalences found in the present study are noticeably higher than those previously reported in beef and dairy farms of Galicia [11, 18–20]. Our data was even higher than that detected in dairy organic farms from north-western Spain, where the risk of parasite infection is particularly high due to outdoor rearing of livestock and legal restrictions on the use of anthelmintics [21]. In contrast, recent data on sheep from Galicia showed a substantially lower prevalence (14%) [34], which is consistent with previous reports in Europe [15, 35]38– [41]. These evidence, together with results of experimental infections [42, 43], may suggest that paramphistomids are better adapted to cattle.

The infection rates recorded in this investigation are among the highest in Europe and similar to those reported in some countries of the British Islands such as Ireland (48.8–53.8%) [22, 38, 44] or Scotland (43.3%) [35]. However, infection rates in cattle across the rest of Europe are noticeably lower; a thorough review of available data suggests that countries located further east tend to report lower prevalence rates. For example, a recent study in western France (Normandy) showed a prevalence of 29.9% [23], although in France, prevalences as high as 50% were recorded in the 1990's [13]. Similar infection rates were found in Belgium (28.8%) [45] and the Czech Republic (29.9%) [46] whereas lower percentages of infection were recorded in cattle from Germany (12.7%) [41] or Italy (10.9%) [39]. The highest prevalences detected in the westernmost part of Europe may be related to its climate, since the abundant rainfall and moderate temperatures throughout the year favour the

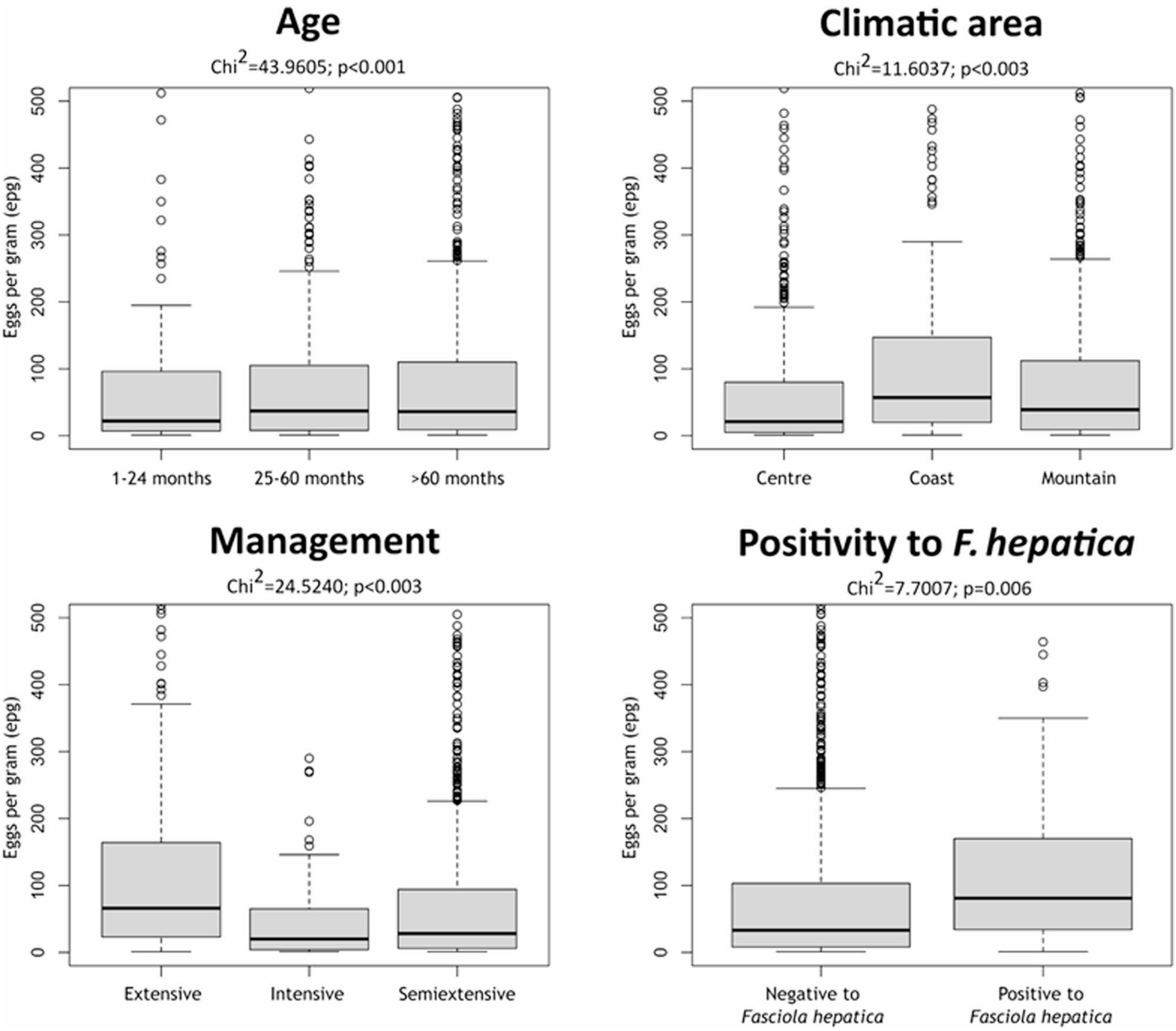

Fig. 3 Box plot representing the distribution of paramphistomid egg shedding according to age, climatic area, management and infection with *F. hepatica*

development of the life cycle of rumen flukes. This climate could enhance the survival of the external stages of rumen flukes in the environment, as they are less exposed to desiccation and extreme temperatures [15]. In this sense, it has been proven that miracidia can infect snails in a temperature range of 1–35 °C [47]. In addition, moderate temperatures throughout the year can extend the period of activity of intermediate hosts [48].

The mean egg shedding of positive animals was 96.6 epg. Comparing egg counts can be very complex, as the sensitivity of the techniques used can lead to marked differences in epg values. However, a good correlation has been demonstrated between the shedding of paramphistomid eggs and the parasite burden in the host's forestomachs, in contrast to that reported for hepatic trematodes [45, 49]. In this respect, the average shedding rate observed in this study can be considered as moderate and consistent with moderate parasite burdens; this intensity of infection does not normally lead to the presence of clinical signs in adult animals, which have good tolerance to paramphistomid infections. However, further studies are needed to unravel their impact on productive parameters such as milk production, fertility or feed conversion ratio. In addition, depending on factors such as the number of animals, presence of intermediate hosts or the available pasture area, even small egg shedding can lead to an increase of the pasture contamination with metacercariae over time, which can lead to the onset of clinical paramphistomidosis in young animals [9].

Regarding risk analysis, the logistic regression revealed that the probability of cattle being infected with rumen flukes was significantly higher in Asturias than in Galicia.

Specifically, higher individual (68.7%), herd (91.2%) and intra-herd (80%) prevalences were found in Asturias than in Galicia (45.6%, 78.6% and 60.2%, respectively). Since both regions have a similar climate, the observed differences may be due to variations in health management practices. In Spain, animal health issues mainly depend on regional governments, leading to substantial differences between regions. In addition, the availability of previous data on cattle in Galicia [11, 18, 20] along with the education and training provided to farmers and veterinarians through numerous informative sessions in this region (Díaz, personal communication) has increased awareness of the presence of paramphistomids, favouring the implementation of more suitable preventive and control measures. In this regard, changes in animal and pasture management were recommended after correct diagnosis such as identifying and fencing those areas representing favourable habitats for intermediate hosts, avoiding young animals sharing grazing areas with adults, as well as including strategic treatments with oxyclozanide.

The risk analysis also revealed a significant and direct relationship between the age of the animals and both the probability of infection and egg shedding, being consistent with previous studies [10, 50–53]. All this data suggests that cattle only develop a partial protective immunity against future infections, at least against juvenile paramphistomids, since clinical cases usually occur in young animals [9] This immunity May prevent the establishment of large numbers of paramphistomids in the duodenum but does not prevent some of them from completing their development. In this regard, the longevity of paramphistomids must be also considered, since their life span can extend up to 10 years, leading to an accumulation of parasites throughout the animal's lifetime [43, 54, 55] and, consequently, to the higher prevalence and egg shedding values found in older animals. Thus, further research is needed to explore the influence of age on rumen fluke infection and the dynamics between these parasites and the host's immune system.

Animals infected with *F. hepatica* had a significant higher risk of testing positive for *C. daubneyi* and shed a significantly higher number of eggs of this paramphistomid. Although this effect on egg shedding had not been previously documented in cattle, it has recently been observed in *F. hepatica* positive sheep from Galicia [34]. Moreover, a similar effect on *F. hepatica* egg shedding had been described in sheep infected by paramphistomids [56]. In Europe, both parasites share their intermediate host, *G. truncatula*. Consequently, if an animal is infected with *F. hepatica*, it is likely to have frequented the same areas where the metacercariae of paramphistomids are primarily found. In addition, it has been demonstrated that *F. hepatica* exerts an immunomodulatory effect on the host that facilitates infection by other bacterial agents [57], so its influence in this regard cannot be ruled out.

The statistical analysis also indicated that animals from farms that did not use scrapers were 76.9 times more likely to be infected with paramphistomids. This factor may be related to the level of professionalization, which is challenging to quantify but clearly influences prevalence rates [58]. In the studied area, the farms using scrapers tend to be more professionalized, and their animals used to spend more time indoors. In addition, faeces collected by the scrapers fall into a slurry pit, where fermentative processes destroy the parasitic forms [59]. Thus, this slurry can be used as fertilizer without contributing to increase the environmental contamination. In any case, further studies are needed to unravel the effect of farm professionalization on the prevalence of rumen flukes.

The differences in egg elimination between climatic areas, particularly between the coastal and the central area, may be due to more favourable conditions for the survival of the external stages of the parasite in coastal areas [60], where winters are milder and humidity is high throughout the year [61]. These conditions enhance the survival of the parasite in the environment, leading to an increased parasite burden in pastures and, therefore, in the animals grazing there.

The greater elimination of eggs observed in extensive farms compared to semi-extensive or intensive farms is probably due to the daily and continuous contact with the parasite [10, 62]; in contrast, cattle from semi-extensive farms have intermittent contact with metacercariae, while those from intensive farms are only exposed to the parasite when fed with fresh grass. In addition, in extensive farming, sanitary control and deworming are more challenging and less frequent, which may result in higher parasite burdens.

Finally, our results suggest that *C. daubneyi* is the only paramphistomid species in northwestern Spain, agreeing with previous studies in both cattle and sheep [11, 34]. Although *Paramphistomum leydeni* has been previously found in cattle, sheep and wild ruminants from different European countries [36, 37, 63, 64], further investigations are needed for determining whether *C. daubneyi* is the only paramphistomid species present in north-western Spain.

Conclusions

Our results reveal a growing trend in the prevalence of paramphistomid infections in cattle from Galicia over the last years, despite the availability of data and ongoing information campaigns. The situation in Asturias is particularly noteworthy, given the lack of prior individual data and the significantly high prevalence rates observed. Consequently, it is imperative to continue monitoring the situation in these regions, enhance awareness among livestock farmers and veterinarians, and carry out farm-specific risk assessments to implement the most effective pharmacological and management interventions.

Abbreviations

AIC	Akaike information criterion
ANOVA	Analysis of variance
BIC	Bayesian Information Criterion
BLAST	Basic Local Alignment Search Tool
epg	Eggs per gram of faeces
ITS-2	Internal transcribed spacer 2
opg	Oocysts per gram of faeces
SD	Standard deviation

Acknowledgements

The authors would like to thank the veterinarians and farmers of the participating farms for their collaboration.

Authors' contributions

Conceptualization: PD, CML; Methodology: CML, PD; Formal Analysis: DGD, CML; Investigation: DGD, PD, AS, SR; Resources: CF, DGD, PD, NMC; Writing original draft: DGD; Writing, revision and Editing: PD, CML, RP, PM; Visualization: DGD; Funding: PM, RP. All authors reviewed and accepted the final manuscript.

Funding

This study has received funding from the Program for consolidating and structuring competitive research groups (ED431C 2019/04 and ED431C2023/16, Xunta de Galicia, Spain).

Data availability

The data supporting the conclusions of this article are included within the article. A more detailed dataset used during the current study is available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

All faecal samples used in this study were collected with the permission of the farm owners. All experimental procedures fully complied with European and Spanish ethics regulations on the protection of animals used for scientific purposes (European Directive 2010/63/EU and Spanish Royal Decree 53/2013) and approved by the ethical committee of the University of Santiago de Compostela.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 2 June 2025 / Accepted: 18 August 2025

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