Calicophoron daubneyi (Paramphistomidae) in slaughtered cattle in Castilla y León (Spain).

M Carmen Ferreras[a*], Camino González-Lanza[a], Valentín Pérez[a], Miguel Fuertes[a], Julio Benavides[a], Mercedes Mezo[b], Marta González-Warleta[b], Javier Giráldez[a], Ana María Martínez-Ibeas[a], Laetitia Delgado[a], Miguel Fernández[a], M Yolanda Manga-González[a]

[(a)] Instituto de Ganadería de Montaña CSIC-ULE. Finca Marzanas. Grulleros. León, SPAIN.

[(b)] Centro de Investigaciones Agrarias de Mabegondo-INGACAL, Xunta de Galicia. Carretera Betanzos-Mesón do Vento, km 7. 15318 Abegondo, A Coruña, SPAIN.

*Corresponding autor:

Mª del Carmen Ferreras,

Instituto de Ganadería de Montaña CSIC-ULE.

Departamento de Sanidad Animal.

Facultad de Veterinaria,

Universidad de León,

Campus de Vegazana, s/n,

24071 León,

Spain

Phone: +34 987291326

Fax: +34 987291194

E-mail: mcfere@unileon.es

Abstract

The prevalence and aetiology of natural paramphistomosis was investigated in cattle slaughtered in the Castilla y León region (Spain) over a 3 year-period. The overall prevalence of positive animals was 6.20%. The parasite burden per animal ranged from 8 to 8005 (median = 144) and the ruminal atrium had the highest parasite burden whereas the ruminal dorsal sac the lowest. The prevalence and parasite burden increased with age while these parameters were lower in cattle under intensive management. *Calicophoron daubneyi* was the only

Paramphistomidae species identified using morphoanatomical, histological and molecular methods in the studied animals.

Keywords

Paramphistomosis, *Calicophoron daubneyi*, cattle, epidemiology

Introduction

Paramphistomosis is a ruminant digestive parasitism caused by different species of Paramphistomidae (Trematoda, Digenea), belonging to several genera (*Paramphistomum*, *Calicophoron* and *Cotylophoron*). It has a worldwide distribution and in tropical and sub-tropical regions is regarded as a highly pathogenic disease (Dorny et al., 2011). In Europe, this parasitism is generally considered as clinically irrelevant, although occasionally weight loss and decreased milk production (Spence et al., 1996; Foster et al., 2008), or even mortality in adult sheep, has been described (Mason et al., 2012).

Castilla y León region, in the northwest of Spain, has the largest cattle population in Spain (over one million). Nevertheless, there is a lack of information about paramphistomosis in domestic ruminants of this area. In Galicia, a geographically close region, *Calicophoron daubneyi* (Dinnik, 1962) was the only paramphistome found in slaughtered cattle (González-Warleta et al., 2013).

The aim of this study was to investigate the prevalence of Paramphistomidae species in abattoir slaughtered cattle in Castilla y León, and determine which species were present.

Materials and methods

Animals

Cattle sampled (total n = 790) were from both mountainous and arable areas, primarily from León, Zamora, Valladolid and Palencia provinces (Northwestern Spain), during the spring-summer seasons of 2010, 2011 and 2012 from an abattoir located in the city of León (Year 1: n = 481; Year 2: n = 173; Year 3: n = 136). Ages ranged from 10 months to 19 years and belonged to both sexes (61.3% females; 38.6% males). The cattle came from 184 farms, 93 of

59 them (50.5%) managed intensively (animals kept indoors and with no access to pasture) and
60 the remaining 91 (49.4%) in a semi-intensive system (at pasture for variable periods of time
61 during the grazing season). Herds were classified, according to their primary purpose, as beef
62 (81.6%) or dairy (18.3%). Detailed information of all the animals and farms was provided by the
63 slaughterhouse and the Livestock Section of the Castilla y León Government.

64 *Sampling*

65 At the slaughterhouse, the rumen, reticulum, omasum, abomasum and proximal duodenum
66 from each animal were examined for the presence of parasites. The rumen and reticulum of all
67 the parasitized cattle were processed for worm recovery.

68 *Parasite count and identification*

69 For each bovid, the presence and number of the parasites in the reticulum and their location(s)
70 in the different anatomical parts of the rumen (rumino-reticular orifice, rumino-reticular fold,
71 ruminal atrium, ventral sac and dorsal sac) was evaluated. A representative number of
72 paramphistomes from each parasitized cattle were fixed in 70% ethanol and 10% neutral-
73 buffered formalin for subsequent identification, while other specimens were frozen individually
74 and stored at -85 ºC until DNA extraction.

75 A total of 472 randomly selected alcohol-fixed paramphistomes were stained with borax carmine
76 and microscopically evaluated as reported previously (Eduardo, 1983). Additionally, a total of
77 200 formalin-fixed paramphistomes were processed for histological examination. Species specific
78 Internal Transcribed Spacer 2 (ITS-2) intergene zone and mitochondrial DNA were amplified for
79 molecular identification of *C. daubneyi* (González-Warleta et al.,2013; Martínez-Ibeas et al.,
80 2013).

81 *Statistical analysis*

82 Animals were classified according to farm intensification level (semi-intensive *vs.* intensive
83 systems), dairy or beef farms and age (<12 months, 12 to 30 months and > 30 months). Effects
84 of these variables on prevalence and parasite burden (logarithmically transformed) were
85 analysed using the non-parametric Kruskal-Wallis test. The strength of the relationship between

prevalence and the above mentioned independent variables were estimated through logistic regression. Proportion of the total worms localized in the reticulum and different anatomical parts of the rumen was subjected to analysis of variance. All statistical procedures were carried out using SAS package (SAS, 2009).

Results

Parasite identification

All the adult parasite specimens studied from the reticula and rumina of infected cattle were identified morphoanatomically and histologically as *C. daubneyi*. The molecular study by ITS-2 confirmed, in all the worms examined, a 410 bp fragment with a nucleotide composition identical (100% homology) to that published for *C. daubneyi* in Gen BankTM (Access No AY790883). Moreover, the studied specimens showed 100% homology with the mtDNA fragment 885 pb of *C. daubneyi* (Gen Bank, Access No JQ815200).

Epidemiological findings

Overall, 49 out of the 790 cattle examined (6.20%) had adult flukes in the rumen and reticulum, and number of parasites per animal ranged from 8 to 8005 (median = 144).

Neither prevalence of natural paramphistomosis nor fluke burden were significantly affected by the production purpose of the animal (beef *vs* dairy), or the breed. However, prevalence rate and fluke burden were significantly higher ($P<0.05$) in semi-intensive (10.04% and 253 respectively) than intensive systems (4.22% and 58).

The age of the animal also showed a significant influence ($P<0.05$) over both the prevalence of infection, from 2.62% (<12 months) to 17.88% (>30 months) and the median worm burden (25 at <12 months; 253 at >30 months). Significant differences ($P=0.001$) were observed also between females (8.71%; n=42) and males (2.27%; n=7).

However, when all the factors were studied together, age was the only independent variable that was significantly correlated with the prevalence of *C. daubneyi* infection (Odds ratio=2.49; 1.55 to 4.902; P=<0.001). The average slaughter age was greater for cattle from semi-intensive than intensive systems (51.4 ± 3.49 *vs* 18.9 ± 1.08 months) and for females compared to males (41.4± 2.38 *vs* 12.0 ± 0.16 months).

When considering the production purpose of the animal, it is worth noting that 417 of the 790 cattle examined (52.7%) came from feedlots and *C. daubneyi* was detected in only 19 of them (4.5%). It should also be mentioned that these feedlot cattle were breeding on pastures during different periods of time.

Parasite burden

The number of fluke was significantly (*P*<0.01) higher in the rumen than in the reticulum (90.81±1.71% *vs* 9.19±1.71%). Within the rumen, they were more numerous in the ruminal atrium (62.93±3.31%) and the ventral sac (16.53±2.15%). Animals from the semi-intensive management system had significantly less parasites in their ruminal atria (*P*<0.005, Table 1). Older animals (>30 months) had the highest fluke burden (*P*<0.05), with an increased number of parasites in the rumino-reticular fold compared to the other groups (Table 1).

Discussion

The present study is the first report of the prevalence and aetiology of naturally acquired paramphistomosis *by C. daubneyi* in slaughtered cattle from the Castilla y León region (Spain).

The overall prevalence (6.20%) was lower than those reported in other areas of Spain or France (González-Warleta et al., 2013; Szmidt-Adjidé et al., 2000), but similar to that observed in Algeria (Titi et al., 2010). Climate has been proposed to influence infection rate in cattle (Titi et al., 2010) and as Castilla y León has a temperate to cold, dry climate this may hinder the speed of the parasite life-cycle. An unexpected finding of this study was the presence of naturally acquired paramphistomosis in cattle from feedlots. This finding, not recorded in other Spanish territories (Arias et al., 2011; González-Warleta et al., 2013), has been also observed in Irish store cattle (Murphy et al., 2008). Possible explanations are if that these animals were infected

while grazing at pasture before their confinement in feedlots or ingestion of contaminated fodder while in the feedlot.

Paramphistomosis infection was found in animals of all age categories, although the prevalence was significantly higher in cattle older than 30 months, suggesting that repeated exposures to the parasite do not confer protection against re-infections. This is in contrast to other studies (Dorny et al., 2011). A higher percentage of females animals were infected which is in agreement with the suggested genetic or hormonal predisposition of females to paramphistomosis (Szmidt-Adjidé et al. 2000; Titi et al., 2010). However, it has to be considered that, in this study, sex was closely related to the age of the animal.

The climate of the region may have contributed to an individual parasite burden lower than those reported in other areas with a more humid and less extreme climates (Szmidt-Adjidé et al., 2000; Arias et al., 2011; González-Warleta et al., 2013). Differences observed according to the management system are probably related to the maintenance at pasture for longer periods of time of animals under a semi-intensive than an intensive system and consequently more exposure to the infective form of the parasite.

The mean worm count was the highest in the ruminal atrium and the lowest in the dorsal sac, in agreement with the findings of González-Warleta et al. (2013). The lower burden in the latter may be related to its scarce mucosal papillae which is due, in turn, to the gas dome of CO_2 and methane present in the area (Tschuor and Clauss, 2008). The long leaf-shaped papillae of the atrium may have had a protective effect for the flukes and the presence of a fluid layer may favour the intake of nutrients by the parasite.

Our results showed that *C. daubneyi* is the only Paramphistomidae species found in naturally infected cattle in the Castilla y León region. This species, although considered as one of the less widespread (Silvestre et al., 2000), has also been found in other regions of Spain (González-Warleta et al. 2013; Martínez-Ibeas et al. 2013), across Europe (Silvestre et al., 2000; Szmidt-Adjidé et al., 2000;Gordon et al., 2013) and Algeria (Titi et al., 2010).

In conclusion, this is the first study of the prevalence of cattle paramphistomosis in Castilla y León region of Spain, and the only species identified was *C. daubneyi.* Data provided may be

helpful to devise further studies about seasonality of infection, habitat of the intermediate host, interactions with other trematodes and appropriate control strategies for paramphistomosis.

Competing interests

The authors declare that they have no competing interests.

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Figure captions.

Fig 1. Adults of *C. daubneyi* present on the reticulum mucosa from an adult cow.

Bar= 1 cm.

264 Table 1. Effect of farm characteristics and age of animals on distribution of parasites in the
265 rumen-reticulum (mean value ± standard error)

	Localization		Intraruminal localization				
	Reticulum	Rumen	Orifice	Ruminal-reticulum fold	Atrium	Ventral sac	Dorsal sac
Type							
Dairy	9.6±4.20	90.4±4.20	2.8±1.028	15.4±4.41	63.2±6.18	16.3±2.52	2.3±1.22
Beef	9.1±1.88	90.9±1.88	9.6±3.08	8.9±2.01	62.9±3.92	16.6±2.69	2.0±0.56
Management system							
Semi-intensive	11.5±2.51	88.5±2.51	6.8±2.34	13.3±3.01	57.1±4.25^{a}	19.9±3.22	2.8±0.83
Intensive	6.3±2.16	93.6±2.16	9.6±4.64	6.7±1.64	70.1±4.88^{b}	12.3±2.52	1.2±0.44
Age							
< 12 months	3.4±1.76^{a}	96.6±1.76^{a}	4.8±4.83	5.9±1.96^{a}	72.5±6.30^{b}	15.5±5.37	1.2±1.23
12 to 30 months	4.1±1.32^{a}	95.9±1.32^{a}	3.2±1.60	3.4±1.34^{a}	76.6±4.00^{b}	14.5±4.60	2.2±0.84
> 30 months	12.6±2.54^{b}	87.4±2.54^{b}	10.7±3.63	14.0±2.76^{b}	55.5±4.44^{a}	17.5±2.83	2.3±0.70

266 ^{a,b,c} Within the same factor and column, means with different letters are significantly different
267 ($P<0.05$)

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Figure 1