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Calicophoron daubneyi (Dinnik, 1962) (Digenea) in beef and dairy cattle in the Czech Republic: prevalence and drug efficacy

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Abstract: A total of 1,724 beef and 2,941 dairy cattle older than one year from 66 beef and 67 dairy farms in the Czech Republic were examined for the presence of rumen and liver fluke eggs in 2019–2022. Out of 227 positive animals, all were positive for paramphistome and five for fasciolid eggs. Molecular analysis of the ITS2 rDNA revealed the presence of *Calicophoron daubneyi* (Dinnik, 1962) and *Fasciola hepatica* Linnaeus, 1758. Faecal egg count (FEC) showed low infection intensity (12 EPG) in animals infected with *F. hepatica* and high variability in *C. daubneyi* infections (2–589 EPG). Efficacy of oxyclozanide, albendazole, ivermectin, and closantel against *C. daubneyi* infection was evaluated at eight beef cattle herds. Faecal samples were collected from all positive animals at 0 and 21days post-treatment. Based on FEC, albendazole, ivermectin and closantel reduced the number of *C. daubneyi* eggs shed by 0–9.9%, with no effect on the number of infected animals. The use of oxyclozanide on two beef farms showed 100% efficacy against *C. daubneyi* and *F. hepatica*. Follow-up examination 5–6 months after drug application showed reinfection of most animals with *C. daubneyi*, but the FEC was significantly lower. The finding of four dairy cows infected with *C. daubneyi* housed in a stable without pasture suggests the possibility of the infection being introduced through roughage.

Keywords: PCR, faecal egg counts, albendazole, ivermectin, oxyclozanide, closantel

Trematodes belonging to the families Fasciolidae and Paramphistomidae are parasites most commonly found in grazing animals in moist habitats that provide suitable conditions for their development in intermediate hosts. Trematodes of the family Paramphistomidae, so-called rumen flukes, are important parasites of wild and domestic ruminants worldwide (Eduardo 1983, Foster et al. 2008, Millar et al. 2017). While mature flukes that inhabit the rumen and reticulum do not usually cause clinical disease (Rolfe et al. 1994, Zintl et al. 2014), immature flukes inhabiting the duodenum can cause hemorrhagic lesions of the mucosal tissues leading to reduced weight and milk production, oedema, and even death of the host (Rolfe et al. 1994, Spence et al. 1996, Millar et al. 2012).

In the past, rumen flukes were thought to be mainly restricted to subtropical and tropical areas. However, in recent decades, a high incidence of rumen flukes has been noted in many temperate European countries. Using molecular tools, the species *Calicophoron daubneyi* (Dinnik, 1962) has been identified as the most common rumen fluke infecting cattle, sheep, and goats in Europe (Arias et al. 2011, Gordon et al. 2013, Ferreras et al. 2014, Martinez-Ibeas et al. 2016).

Calicophoron daubneyi has a life cycle similar to *Fasciola hepatica* Linnaeus, 1758 and shares the same intermediate host, the snail *Galba truncatula* (Müller). Co-infection by both flukes in the final host is possible (Jones et al. 2017, Forstmaier et al. 2021). The occurrence of paramphistome flukes in the Czech Republic does not seem to be rare, but few studies are available. *Paramphistomum cervi* (Zeder, 1790), *Calicophoron microbothrium* (Fischoeder, 1901), and *Paramphistomum ichikawai* Fukui, 1929 have been reported to occur in domestic and wild ruminants (Kotrlá and Chroust 1978, Kotrlá and Kotrlý 1982, Pavlásek 1995, Bazsalovicsová et al. 2010, Oberhauserová et al. 2010).

Although the occurrens of *C. daubneyi* has recently been described in beef cattle in the Czech Republic (Červená et al. 2022), no studies on trematode burden and treatment efficacy have been conducted in the Czech Republic. Therefore, this aim of this study was to describe the prevalence of rumen flukes in beef and dairy cattle in the Czech Republic and to evaluate the efficacy of anthelmintics (albendazole, ivermectin, closantel and oxyclozanide) against this parasite in naturally infected animals.

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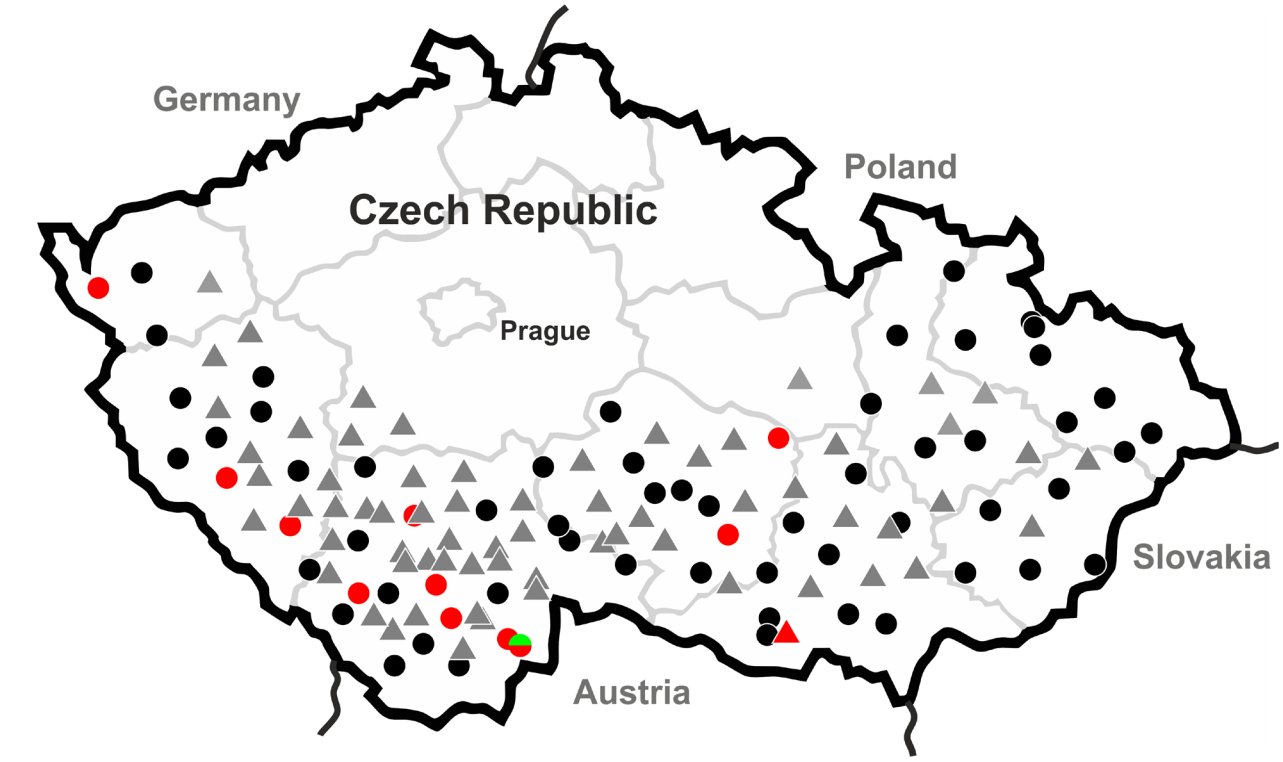

Fig. 1. Location of beef (circles) and dairy (triangles) farms screened in the Czech Republic. The red and green colour indicates farms with the occurrence of *Calicophoron daubneyi* (Dinnik, 1962) and *Fasciola hepatica* Linnaeus, 1758, respectively. Only the ID of farms where flukes were found are listed.

MATERIALS AND METHODS

In 2019 and 2022, faecal samples were collected from 1,724 beef and 2,941 dairy cattle *Bos primigenius* f. *taurus* (Linnaeus) older than one year, on 66 beef and 67 dairy farms in the Czech Republic (Figure 1). Faecal material was collected during the project "Monitoring of parasites in beef and dairy cattle in the Czech Republic" at the Institute of Parasitology, Biology Centre of the Czech Academy of Sciences and Faculty of Agriculture and Technology, University of South Bohemia in České Budějovice.

Samples were collected by the project team in collaboration with livestock farmers during visits to the selected farms. All herds whose owners were willing to cooperate in the project were included in the study. Sampling in the central and northern parts of Bohemia was excluded for logistical reasons. At least 30 g of faeces were collected from each animal directly from the rectum or immediately after defecation to protect against contamination and misidentification, labelled with the animal ID (on its ear tag), kept cool at 4–8°C in an airtight sterilised container without fixative, delivered to the laboratory, and examined as described below within 48 h post collection.

The presence of fluke eggs and faecal egg count (FEC) in preparations were carried out after sedimentation according to Thienpont et al. (2003) with following modification: a total of 4 g of faeces were homogenised in 100 ml cold tap water, loaded onto the system of 600, 300 and 63 µm metal sieves and filtered for 5 minutes with a stream of cold tap water. The suspension collected on the 63 µm sieve was transferred to a 50 ml falcon tube and spun for 5 minutes at 1000 g. The supernatant was removed and screened for the presence and number of eggs under 40 × magnification. Rumen fluke eggs were identified based on their morphological characteristics (Arias et al. 2011, Gordon et al. 2013).

Total genomic DNA (gDNA) was extracted from approximately 100 paramphistome or fasciolid eggs collected from three randomly selected positive animals per farm per in each sampling term (a total 75 samples) using a Pasteur pipette by bead disruption for 60 s at 5.5 m/s using 0.5 mm glass beads in a Fast Prep®24 Instrument (MP Biomedicals, Santa Anna, CA, USA) followed by isolation/purification using a commercially available kit in accordance with the manufacturer's instructions (Exgene™ Stool DNA mini, GeneAll Biotechnology Co. Ltd., Seoul, Korea). Purified DNA was stored at -20°C prior to being used for PCR.

PCR protocols were used to amplify the internal transcribed spacer 2 (ITS-2 rDNA) using previously published protocols (Itagaki et al. 2003). The PCR mixtures contained 2 µl of gDNA, 10 µl of AmpONE™ HS-Taq premix (GeneAll Biotechnology), 200 nM of each primer, and molecular grade water up to a volume of 20 µl. Negative (molecular grade water) and positive (DNA of *Paramphistomum cervi*) controls were included in each PCR amplification. PCR products were visualised in a 2% agarose gel stained with ethidium bromide (0.5 mg/ml) and prepared with TBE (50 mM Tris, 45 mM boric acid, and 0.5 mM EDTA; Invitrogen, USA).

Sequencing was carried out in both directions using an ABI 3130 Sequence Analyser (Applied Biosystems, Foster City, CA, USA). Purified secondary PCR products were sequenced in both directions using the secondary PCR primers and the BigDye 1 Terminator V3.1 at a commercial company (SeqMe, Dobříš, Czech Republic). The nucleotide sequences were edited using Chromas Pro 2.4.1 software (Technelysium, Pty, Ltd., South Brisbane, Australia), verified by BLAST analysis (https://blast.ncbi.

Table 1. List of beef (B) and dairy (D) farms investigated in this study with the number of animals tested and found positive for *Calicophoron daubneyi* (Dinnik, 1962) [CD] and *Fasciola hepatica* Linnaeus, 1758 [FH] on each farm. Positive farms are highlighted.

Farm ID	No. of screened/positive for CD/FH	Farm ID	No. of screened/positive for CD/FH	Farm ID	No. of screened/positive for CD/FH	**Farm ID**	**No. of screened/positive for CD/FH**
			Beef farms				
B1	21/0/0	**B18**	10/0/0	**B35**	99/0/0	**B52**	43/0/0
B2	50/0/0	**B19**	35/0/0	**B36**	**12/10/0**	**B53**	45/0/0
B3	80/0/0	**B20**	**22/22/0**	**B37**	34/0/0	**B54**	23/0/0
B4	13/0/0	**B21**	30/0/0	**B38**	56/0/0	**B55**	26/0/0
B5	36/0/0	**B22**	12/0/0	**B39**	23/0/0	**B56**	**17/17/0**
B6	10/0/0	**B23**	84/0/0	**B40**	41/0/0	**B57**	26/0/0
B7	**35/34/0**	**B24**	49/0/0	**B41**	45/0/0	**B58**	42/0/0
B8	77/0/0	**B25**	21/0/0	**B42**	**27/27/0**	**B59**	**40/40/5**
B9	30/0/0	**B26**	17/0/0	**B43**	19/0/0	**B60**	38/0/0
B10	22/0/0	**B27**	23/0/0	**B44**	25/0/0	**B61**	56/0/0
B11	**9/5/0**	**B28**	10/0/0	**B45**	67/0/0	**B62**	108/0/0
B12	16/0/0	**B29**	11/0/0	**B46**	34/0/0	**B63**	24/0/0
B13	11/0/0	**B30**	20/0/0	**B47**	87/0/0	**B64**	56/0/0
B14	23/0/0	**B31**	**30/30/0**	**B48**	24/0/0	**B65**	72/0/0
B15	**25/23/0**	**B32**	54/0/0	**B49**	**13/12/0**	**B66**	56/0/0
B16	13/0/0	**B33**	**12/3/0**	**B50**	27/0/0		
B17	15/0/0	**B34**	40/0/0	**B51**	36/0/0		
			Dairy farms				
D1	23/0/0	**D18**	45/0/0	**D35**	42/0/0	**D52**	42/0/0
D2	45/0/0	**D19**	56/0/0	**D36**	20/0/0	**D53**	50/0/0
D3	36/0/0	**D20**	79/0/0	**D37**	23/0/0	**D54**	35/0/0
D4	23/0/0	**D21**	24/0/0	**D38**	45/0/0	**D55**	38/0/0
D5	89/0/0	**D22**	102/0/0	**D39**	64/0/0	**D56**	41/0/0
D6	76/0/0	**D23**	**56/4/0**	**D40**	71/0/0	**D57**	66/0/0
D7	54/0/0	**D24**	23/0/0	**D41**	26/0/0	**D58**	23/0/0
D8	23/0/0	**D25**	48/0/0	**D42**	52/0/0	**D59**	27/0/0
D9	56/0/0	**D26**	96/0/0	**D43**	43/0/0	**D60**	36/0/0
D10	98/0/0	**D27**	33/0/0	**D44**	40/0/0	**D61**	67/0/0
D11	124/0/0	**D28**	41/0/0	**D45**	29/0/0	**D62**	40/0/0
D12	47/0/0	**D29**	27/0/0	**D46**	23/0/0	**D63**	28/0/0
D13	9/0/0	**D30**	35/0/0	**D47**	45/0/0	**D64**	13/0/0
D14	34/0/0	**D31**	63/0/0	**D48**	17/0/0	**D65**	19/0/0
D15	23/0/0	**D32**	43/0/0	**D49**	16/0/0	**D66**	30/0/0
D16	45/0/0	**D33**	51/0/0	**D50**	19/0/0	**D67**	45/0/0
D17	83/0/0	**D34**	23/0/0	**D51**	23/0/0		

nlm.nih.gov/Blast.cgi), and aligned with reference sequences from GenBank using BioEdit 7.0.5.3 (Hall 1999). The alignments were then end-trimmed and ambiguous columns were eliminated. Phylogenetic analyses were performed and the best DNA/Protein phylogeny models were selected using MEGA software (Guindon and Gascuel 2003, Tamura et al. 2013). Phylogenetic trees were inferred by maximum likelihood (ML). Bootstrap support for branching was based on 1,000 replications.

This study was performed as part of the routine parasitological control of animals and no ethical permission was required according to the laws of the Czech Republic (Act No. 246/1992 Coll., on the protection of animals against cruelty).The faecal egg count reduction test (FECRT) was used to evaluate the efficacy of anthelmintics used on farms with fluke infection. Individual faecal samples were collected at day 0 before treatment and day 21 post-treatment (Malrait et al. 2015, Fairweather et al. 2020). All treated animals in each herd were screened. FECRT was expressed as the difference in arithmetic means between pre-treatment and post-treatment EPG levels with 95% confidence limit. The differences in FEC between pre- and post-treatment were assessed using the nonparametric Manne-Whitney test with significance $P < 0.05$ in R 4.0.0. (R Core Team 2019). Each host serves as its own control for FECRT.

A total of four different anthelmintics or combinations thereof were used on the farms surveyed (Table 2). The Ivomec (Merial, Lyon, France) was administered subcutaneously at the recommended dose 200 mg of ivermectin per kg body weight (BW); Aldifal 100 (Mikrochem, Pezinok, Slovakia;) was administered orally at recommended dose 10 mg of albendazole per kg BW; the Closamectin (Norbrook Laboratories Limited, Newry, Northern Ireland) was administered pour-on at recommended dose 0.2 mg of ivermectin and 5 mg closantel BW and Distocur (Merial, Lyon, France) was administered orally at the recommended dose 10 mg of oxyclazanide per kg BW. The using of the Distocur treatment was permitted by the Institute for State Control of Veterinary Biologicals and Medicines bulletin (No. 1, 2016). The treatment was performed during the veterinary procedures, the animals were fixed and weighed before the administration of the drug. The therapeutic dose of the drug was calculated based on the weight of the animal.

RESULTS

Microscopic examination of samples from 1,724 beef and 2,941 dairy cattle revealed the presence of rumen fluke eggs in 227 animals. Of these, 223 were detected on 11 of the 66 beef farms (18.2%), while four were found on one (D23)

Table 2. Faecal egg counts (FEC) of *Calicophoron daubneyi* (CD) and *Fasciola hepatica* (FH) on beef farms on days 0 and 21 after treatment by Aldifal 100 (ALD, in dose of 10 mg of albendazol per kg of body weight), Ivomec (IVC, in dose of 0.2 mg Ivermectin per kg of body weight), Closamectin (CLO, in dose of 0.2 mg of ivermectin and 5 mg closantel per kg of body weight), and Distocur (DIS, in dose 10 mg of oxyclozanide per kg of body weight) and efficiency of treatment expressed as the percentage decrease in excreted eggs after treatment. Statistically significant differences between pre-treatment and post-treatment values were assessed by ManneWhitney test (*P < 0.05). Min - the smallest amount of eggs shed per gram of faeces; Max - the greatest amount of eggs shed per gram of faeces; CI – confidence interval; ND – not defined

Farm ID	Screening term	Used anthel-mintics	Fluke species	Day 0				Day 21				Efficiency (%) (CI 95%)
				Number of treated / positive animals	FEC			Number of treated / positive animals	FEC			
					Mean ± SD	Min	Max		Mean ± SD	Min	Max	
B7	Fall 2020	IVC	CD	35/33	92 ± 89	5	405	35/33	91 ± 85	12	357	0.9 (0–37.7)
	Spring 2021	IVC	CD	35/34	101± 92	12	409	35/34	100 ± 98	14	400	0.4 (0–37.3)
B11	Spring 2021	–	CD	9/5	10 ± 12	9	34	9/5	9 ± 10	12	25	6.7 (0–68.2)
B15	Spring 2021	IVC	CD	25/23	26 ± 16	9	70	25/23	27 ± 15	11	56	0 (ND)
	Fall 2021	IVC	CD	25/23	27 ± 16	11	67	25/23	26 ± 17	12	76	3.8 (0–32.4)
B20	Spring 2021	CLO	CD	22/22	52 ± 29	9	109	22/22	54 ± 24	23	100	0 (ND)
	Fall 2021	CLO	CD	22/22	51 ± 21	21	99	22/22	48 ± 21	23	111	4.1 (0–25.8)
B31	Fall 2020	IVC	CD	30/30	18 ± 10	4	45	30/30	21 ± 8	6	41	0 (ND)
	Spring 2021	IVC	CD	30/30	23 ± 10	7	48	30/30	23 ± 9	7	43	1.7 (0–21.3)
B33	Spring 2021	–	CD	12/3	3 ± 5	7	14	12/3	3 ± 7	8	23	–
	Fall 2021	–	CD	12/3	5 ± 11	3	34	12/3	4 ± 7	12	20	–
B36	Spring 2021	–	CD	12/8	13 ± 14	2	43	12/8	13 ± 13	4	37	–
	Fall 2021	–	CD	12/10	15 ± 14	3	45	12/10	14 ± 11	5	36	–
B42	Fall 2020	ALD	CD	27/27	144 ± 126	23	589	27/27	130 ± 100	27	478	9.9 (0–43.1)
	Spring 2021	ALD	CD	27/27	135 ± 98	28	400	27/27	136 ±102	24	467	0 (ND)
B49	Spring 2021	IVC	CD	13/12	25 ± 16	9	51	13/12	24 ± 16	11	54	1.3 (0–41.5)
	Fall 2021	IVC	CD	13/12	28 ± 14	17	48	13/12	27 ± 13	13	45	2.7 (0–33.9)
B56	Fall 2020	IVC	CD	17/17	116 ± 84	23	265	17/17	111 ± 70	37	245	5.0 (0–40.8)
	Spring 2021	DIS	CD	17/17	113 ± 75	34	356	17/0	0 ± 0	0	0	100 * (ND)
	Fall 2021	DIS	CD	17/12	29 ± 25	12	89	17/0	0 ± 0	0	0	100 * (ND)
B59	Fall 2020	CLO	CD	40/40	76 ± 39	13	150	40/40	76 ± 48	17	149	0 (ND)
	Spring 2021	DIS	CD	40/40	78 ± 48	23	124	40/0	0 ± 0	0	0	100 * (ND)
			FH	40/5	6 ± 4	2	12	40/0	0 ± 0	0	0	100 * (ND)
	Fall 2021	DIS	CD	40/38	59 ± 28	21	109	40/0	0 ± 0	0	0	100 * (ND)
			FH	40/0	0	0	0	40/0	0 ± 0	0	0	ND

of the 67 dairy farms (1.4%; Table 1). In addition, a mixed infection of rumen and liver flukes was detected in five animals on farm B59 (Table 1). Three faecal samples positive for rumen or liver fluke eggs were randomly selected from each positive herd within each sampling and genotyped. All 75 samples were successfully amplified and sequenced. Molecular analyses of the gene encoding ITS-2 rDNA recovered from samples with rumen fluke eggs were identical to each other and showed 100% homology with the ITS-2 region of *C. daubneyi* (GenBank accession no. KP201674). Sequences of the ITS-2 region of *F. hepatica* recovered from beef cattle in this study were identical to each other and the sequence in GenBank (accession no. MF678650).

Within-herd *C. daubneyi* prevalence was 12.9% (233/1,724) on beef and 0.1% (4/2,941) on dairy farms. At the farm level, the prevalence of *C. daubneyi* on the majority of positive farms (9/12) was above 80% (Table 2). The average number of *C. daubneyi* eggs shed per gram of faeces varied among farms, ranging from 3 to 144 EPG. The highest FEC (589 EPG) was detected on farm B42, where the highest mean EPG was also found (Table 2). There was a significant difference in FEC in animals with a mix of *F. hepatica* and *C. daubneyi* infection: 5 vs 53 EPG, respectively.

The efficacy of anthelmintics used against *C. daubneyi* and *F. hepatica* was monitored on eight out of 11 farms with two to three consecutive applications at an interval of 5–6 months between applications (Table 2). Anthelmintics were not used on three farms (B11, B33 and B36); therefore, these breeds were not included in the evaluation of anthelmintic efficacy (Table 3). Ivomec (Merial, Lyon, France) was used on five farms (B7, B15, B31, B49 and B55). Treatment efficacy against *C. daubneyi* was not effective, efficacy ranging from 0 to 5% (P > 0.05, Table 2). Similar results were observed with the use of Aldifal 100 and Closamectin. Consistent with the low efficacy on FEC, there was no reduction in the number of infected animals after treatment. Distocur (Merial, Lyon, France; 10 mg of oxyclazanide BW) was used on two farms (Table 2). Coprological results 21 days after application showed 100% efficacy on *F. hepatica* and *C. daubneyi* (Table 2). To further investigate the efficacy of Distocur, the animals on these farms were re-examined six months after application of this anthelmintic. Results showed that no animal was positive for *F. hepatica* and that the FEC and number of *C. daubneyi* infected animals were reduced on both farms (Table 2, P < 0.05).

DISCUSSION

The territory of the Czech Republic is historically associated with the occurrence of rumen flukes (*Paramphistomum cervi*, *P. ichikawai* and *Calicophoron*

microbothrium) and *Fasciola hepatica* in grazing domestic and wild ruminates (Hovorka 1963, Kotrlá and Chroust 1978, Kotrlá and Kotrlý 1982, Pavlásek 1995). However, no systematic research has been conducted in the last 20 years. In agreement with a recent study by Červená et al. (2022), we found that *Calicophoron daubneyi* is the predominant fluke species parasitising cattle in the Czech Republic. The percentage of positive farms in this study is comparable to previous studies conducted in similar regions. Similar differences in the occurrence of rumen and liver flukes among regions have been reported in the Netherlands, Germany, the UK, Italy, Ireland, and Spain (Cringoli et al. 2004, Martinez-Valladares et al. 2013, Jones et al. 2017, Ploeger et al. 2017, Munita et al. 2019, Garcia-Dios et al. 2020, Bosco et al. 2021, Fenemore et al. 2021, Forstmaier et al. 2021, Alstedt et al. 2022).

In contrast, the study by Červená et al. (2022) showed a significantly higher percentage of positive farms (63%). The difference between these studies may be influenced by the regions in which the studies were conducted. Approximately 50% of the positive farms in the study of Červená et al. (2022) were in regions of the Czech Republic that were not included in the present study. The solely distribution of *C. daubneyi* in the Czech Republic at the expense of species of the genus *Paramphistomum* Fischoeder, 1900 may be due to the availability of intermediate hosts. While the intermediate hosts of *Paramphistomum* spp. are planorbid aquatic snails, *C. daubneyi* is adapted to *Galba truncatula* and other lymnaeid snails (Vignoles et al. 2017), which are more abundant in regions of the Czech Republic where beef farms are mostly located (Červená et al. 2022).

However, this should mean that *F. hepatica*, which develops in the same intermediate hosts, will be widely distributed at the same time and locations. In addition, experimental studies have shown that *C. daubneyi* and *F. hepatica* can co-infect the same host and that *F. hepatica* cercariae released from an intermediate host are twice as abundant as those of *C. daubneyi* (see Vignoles et al. 2017). While many recent studies in Europe have revealed a high occurrence of *F. hepatica* in domestic ruminants (Kozlowska-Loj 2011, Mazeri et al. 2017, Forstmaier et al. 2021, Opsal et al. 2021), in agreement with previous studies performed in the Czech Republic, we found a low occurrence of this fluke on the screened farms (Zmunda and Chroust 2002, Kváč 2004, Červená et al. 2022).

Most anthelmintics, such as triclabendazole, albendazole, ivermectin and closantel, that are commonly used against *F. hepatica* are not effective against rumen flukes (Probert et al. 1981, Rolfe and Boray 1987, 1988, O'Shaughnessy et al. 2018, Fenemore et al. 2021). This could explain the low number of detected cases of *F. hepatica* infection in our study. A total of eight out of the 11 farms (72%) where flukes were detected used anthelmintics that are effective against *F. hepatica* but ineffective against *C. daubneyi*. Apart from two farms where oxyclozanide was used repeatedly, this anthelmintic was not used on any other farm in this study. In agreement with previous studies in cattle and sheep (Paraud et al. 2009, Sanabria et al. 2014, Delafosse 2022), the use of oxyclozanide was shown to be very effective. In all cases we monitored, the treatment had a 100% effect on adult flukes and prevented the shedding of eggs into the environment. Although *C. daubneyi* infection occurred in treated animals 5–6 months after treatment, there was a significant decrease in FEC and the number of infected animals. Repeated occurrence may be due to reinfection or the fact that oxyclozanide is effective only against adult flukes.

Access to pasture appears to be a critical prerequisite for the occurrence of fluke in breeding animals. While beef cattle graze almost year-round (depending on the climate zone), dairy cattle are more often housed indoors and have limited or no access to pasture. We found infection with *C. daubneyi* in four cows on a dairy farm where the animals had no access to pasture and were fed roughage (silage, hay) and substitutes. Since the metacercariae of *F. hepatica* remain viable in dried hay for 50 days (Enigk and Hildebrandt 1964), we speculate that hay was the source of *C. daubneyi* infection in the dairy cows that were not grazed. This finding suggests that the presence of this fluke in wild ruminants served as a reservoir, as well as the possibility of roughage contamination.

The results of this study show a dominant prevalence of *C. daubneyi* and a low prevalence of *F. hepatica* in beef cattle in the Czech Republic, which is related to the anthelmintics commonly used. Oxyclozanide is one of the few effective anthelmintics against *C. daubneyi* and its wider use could lead to a reduction in the incidence of this fluke as well as *F. hepatica* on beef farms. However it should be noted that oxyclozanide is not currently registered for veterinary use in the Czech Republic.

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