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RESULTS OF PARASITOLOGICAL MONITORING OF BEEF CATTLE HERDS IN THE CZECH REPUBLIC, WITH FIRST DESCRIPTION OF THE OCCURRENCE OF INVASIVE RUMEN FLUKE *CALICOPHORON DAUBNEYI* IN BEEF HERDS

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Abstract

Dynamic changes in the farming practices, growing numbers of beef farmers and geographic distribution of beef cattle herds in the Czech Republic impact on helminth parasites of cattle and vice versa. We summarize in a brief form results of parasitological examination of samples collected by farmers from range of beef cattle farms across the whole Czech Republic in order to provide baseline data for parasite control and to report occurrence of an invasive rumen fluke *Calicophoron daubneyi* in the country.

Keywords: helminths, rumen fluke, *Calicophoron daubneyi*, Czech Republic, beef cattle

INTRODUCTION

Together with other infections, parasitic diseases and mitigation of their impact play an important role in health management of grazing cattle worldwide. Similar to other domestic herbivores on pastures, cattle host a broad array of parasitic protists and helminths, some of which significantly impact on the animal health and, as a consequence, on the economic aspects of the beef production. Changing landscape ecology, changing farming practices and growing demand for organic products, together with a climate change and intensified transboundary movement of animals form a background for dynamic changes in spectrum, abundance and importance of parasites in cattle (i.a. Beesley *et al*., 2017; Skuce *et al*., 2013). Recent emergence and spread of bovine besnoitiosis and rumen fluke *Calicophoron daubneyi* in Europe (Álvarez-García *et al*., 2013; Huson *et al*., 2017) can serve as a prominent example of these changes. Also, the emergence and spread of anthelmintic resistance further stresses a need for accurate and continuing surveillance of parasites in beef cattle herds and for effective control and prophylactic measures (Chartier *et al*., 2020; Vercruysse *et al*., 2018). Regardless growing tendencies towards “precision livestock farming (PLF)” based on demanding continuous real-time monitoring of health, welfare,

production, of individual animals (e.g. Berckmans, 2017), regular screening for parasites detectable in feces remains a pillar for helminth control in extensive pastoral livestock systems.

According to data from the Czech Statistical Office, a yearly total of 1,340,040 cattle was bred in the Czech Republic as of 31 December 2020. There were 559,661 cows in total from which dairy cows accounted for 61% and the share of beef cattle reached 39% (Czech Statistical Office). While dairy cow inventories have steadily decreased, the beef cattle comprise the only cattle category with increasing numbers over the long term. In 2020, there were a total of 226,004 suckler cows, which corresponds to an increase of 600%, compared to 38,000 suckler cows in 1996 (Czech Statistical Office). Massive imports of beef cattle (heifers) were realized mainly in the 1990s with governmental support. Currently, there are about 12 breeds of beef cattle, however, until 1990, the Hereford cattle was the only beef cattle bred in the Czech Republic in pure blood form (Pozdíšek *et al*., 2004). Since 1990, there was a gradual increase of imports of breeding heifers of beef breeds from abroad. The first purchases were realized with the help of subsidized interest-free loans, later state support for the purchase of genetic material from abroad was established. At first, the animals were imported in from Hungary (Charolais, imported in 1990), later mainly from France (Charolais, Limousine, Blonde D'Aquitaine and others) partly from Canada (Aberdeen Angus, Simmental) or from Denmark (Aberdeen Angus, Simmental). Other imports of beef cattle were from Scotland (Highland), Austria (Aberdeen Angus, Galloway, Highland) and from Germany (Aberdeen Angus, Galloway, Highland). Currently, the beef cattle breeding is proving to be a good choice for grassland management in mountain and highland areas. Beef breeds of cattle are in the Czech conditions highly adaptable and able to achieve adequate results of growth indicators (Kynkalová, 2009).

In comparison to other European countries, which have, however, a higher cattle production, very low attention was paid to cattle parasites in the Czech Republic. The same way, cattle parasites are understudied in Czechia in comparison with small ruminants and horses (e.g. Bodecek *et al*., 2018; Chroust, 1998; Kyriánová *et al*., 2017; Langrová *et al*., 2008). Published studies only focused on monitoring of cattle parasites in selected regions and none of them described the situation at the whole country level. Overall, gastrointestinal parasites comprising protists such as *Eimeria*, *Cryptosporidium* and *Giardia* and helminthes - liver and rumen flukes, tapeworms, gastrointestinal and lung nematodes (mostly strongylids) were reported (Chroust, 2006; Hromadová, 2012; Kváč *et al*., 2011; Leontovyč *et al*., 2014; Kubelka, 2016; Pavlásek, 1995). Strongylid nematodes dominated by the genus *Ostertagia* are the most prevalent cattle parasites in the Czech Republic and attracted previously an attention (Chroust, 1982, 2006). Published analyses focus on ovoscopic and larvoscopic observations, quantitative examination and determination of infectious larvae occurrence at pastures (Chroust, 2006; Kubelka, 2016). Further, coccidia of the genus *Eimeria* are often found with the highest prevalence and infection intensity in calves aged around two months to half a year (Chroust, 1966, 2006). The rumen flukes of the family Paramphistomidae (Chroust, 1964), are occasionally causing enteritis and anemia with consequent economic losses. Studies addressing the parasitic nematode abundance and diversity are essential part of measures preventing further spread of parasitic helminths to new localities and emergence of anthelmintic resistance in livestock including the beef cattle (Babják *et al*., 2018; Rose Vineer *et al*., 2020; Vadlejch *et al*., 2014).

The farming practices, numbers of beef farmers and geographic distribution of beef cattle herds in the Czech Republic significantly changed in past decades. The aim of presented paper is to summarize in a brief form results of parasitological examination of samples collected by farmers from range of beef cattle farms across the whole Czech Republic in order to provide baseline data for parasite control and to report occurrence of an invasive rumen fluke *Calicophoron daubneyi* in the country.

MATERIALS AND METHODS

The Project Rationale

Presented material was collected during the project "Monitoring of strongylids involved in helminthiases of beef cattle in the Czech Republic and analysis of anthelminthic resistance and diversity" at the CEITEC VETUNI, University of Veterinary Sciences Brno, State Veterinary Institute Jihlava and Czech Beef Breeder Association. Project mission is to provide country-wide analysis of beef cattle parasites and subsequent analysis of anthelmintic resistance in selected herds. One of the project's goals is active participation of farmers in the campaign (citizen participatory science) and website-based knowledge building on cattle helminthiases and the anthelmintic resistance. Indeed, the major part of the samples was collected by the farmers themselves, the project team also collected samples during the visits of selected farms. Each farmer enrolled in the study received a package with equipment for sample collection and sampling methodology. Samples were collected as fresh as possible from the pastures, barns or directly from rectum during regular veterinary interventions. Samples were immediately transported to the project laboratories or sent by farmers using commercial parcel delivery services. The results of parasitological examination were sent to the farms using project website system.

Coproscopic Examination

All fecal samples were processed by modified Sheather's flotation, simple sedimentation and Vajda's larvoscopy. For each method, a walnut-size aliquot of feces was used. For the flotation, the feces were mixed with ca. 10 ml of water using mortar and pestle and resulting homogenate was sieved to a 15 ml centrifuge tube. After centrifugation (3 min at 2,000 rpm), the supernatant was decanted, Sheather's sugar flotation solution (specific gravity 1.3) was added to the fecal sediment in the tube and sample was homogenized using a vortex. The homogenate was centrifuged again for 3 min at 2,000 rpm. The surface layer with parasite stages was transferred to a slide with a wire loop, covered with a slip and examined under a microscope to detect parasite stages (Deplazes *et al.*, 2016; Foreyt, 2002). For the sedimentation, the feces were mixed with ca. 30 ml of water, homogenized as described in the flotation method and sieved to a 50 ml beaker. After 12 min, the supernatant was decanted and the beaker with fecal sediment was filled up again with water. This procedure was repeated every 10 min, until the supernatant was clean. After the last sedimentation, the remaining 1–2 ml of the fecal sediment was poured to a small Petri dish and examined under a microscope to detect trematode eggs (Deplazes *et al.*, 2016; Foreyt, 2002). Finally, for larvoscopy by Vajda's method, the feces were wrapped in a ca. 5 × 5 cm square of gauze making a small package, and placed in a small Petri dish with water. After 30 min, the feces were removed and the dish was examined for presence of lungworm larvae (Deplazes *et al.*, 2016; Foreyt, 2002). Olympus BX41 microscope was used for examination of all samples. Photographs were taken on an Olympus BX53 equipped with DP73 digital camera. Aliquot of ca. 5 g of selected fecal samples was stored at -20 °C to create a biobank of material for subsequent DNA extraction.

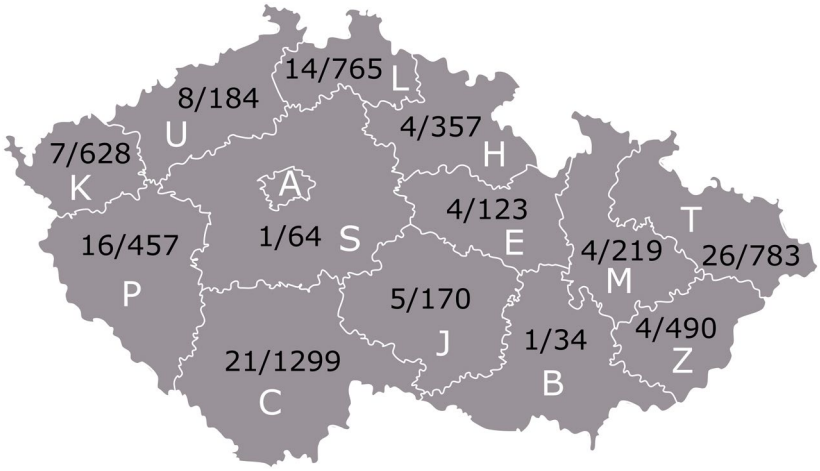
DNA Extraction, PCR and Sequence Analyses

Fecal samples from three different regions (C = South Bohemian, K = Karlovy Vary, U = Ústí nad Labem) where paramphistomid eggs were observed, were used for whole DNA extraction using the DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany) with following modifications: (i) homogenizing samples on the vortex was extended to 30 min, (ii) incubation with C2 solution was extended to 30 min and (iii) incubation with C3 solution was extended to over-night. The DNA was eluted to 100 µl of elution buffer included in the kit. Second internal transcribed spacer region (ITS-2) was amplified in a total volume of 25 µl PCR mixture containing 12.5 µl of PCRBIO Taq Mix Red (PCR Biosystems Ltd., London, UK), 1.25 µl of 10 µM each GA1 (5'-AGAACATCGACATCTTGAAC-3') and BD2 (5'-TATGCTTAAATTCAGCGGGT-3') primers (Anderson and Barker, 1998; Lotfy *et al.*, 2010; Luton *et al.*, 1992), 2 µl of the eluted fecal DNA and 8 µl of PCR grade dH_2O. Cycling conditions were as follows: initial denaturation 1 min at 95 °C followed by 36 cycles of 15 s at 95 °C denaturation, 15 s at 55 °C annealing and 7 s at 72 °C extension with final extension 2 min at 72 °C. The products were separated by electrophoresis in 1% agarose gel stained with Midori Green Advance (Nippon Genetics, Düren, Germany) and visualized on a transilluminator. The bands of expected size were cut off the gel and purified by PCR Cleanup kit (Geneaid Biotech Ltd, New Taipei, Taiwan). Products were sequenced in both directions using the amplification primers at Macrogen Europe (Amsterdam, Netherlands). Sequences were checked, trimmed manually, and assembled in Geneious Prime 2021.0.1 (http://www.geneious.com) and checked against BLAST (Altschul *et al.*, 1990). Sequences of other paramphistomid trematodes ITS-2 were downloaded from GenBank, aligned with our sequences using Clustal Omega implemented in Geneious Prime. The maximum-likelihood phylogenetic tree was calculated by IQ-TREE (Trifinopoulos *et al.*, 2016). Sequences of *Ogmocotyle capricorni* and *O. sikae* were used as an outgroup. The most suitable model was chosen by ModelFinder (Kalyaanamoorthy *et al.*, 2017) implemented in IQ-TREE based on the highest Bayesian information criterion scores and weights (BIC). The tree topology was tested by 1000 replicates of ultrafast bootstrap (Minh *et al.*, 2013) and Shimodaira-Hasegawa (SH)-like approximate likelihood ratio test (Anisimova *et al.*, 2011).

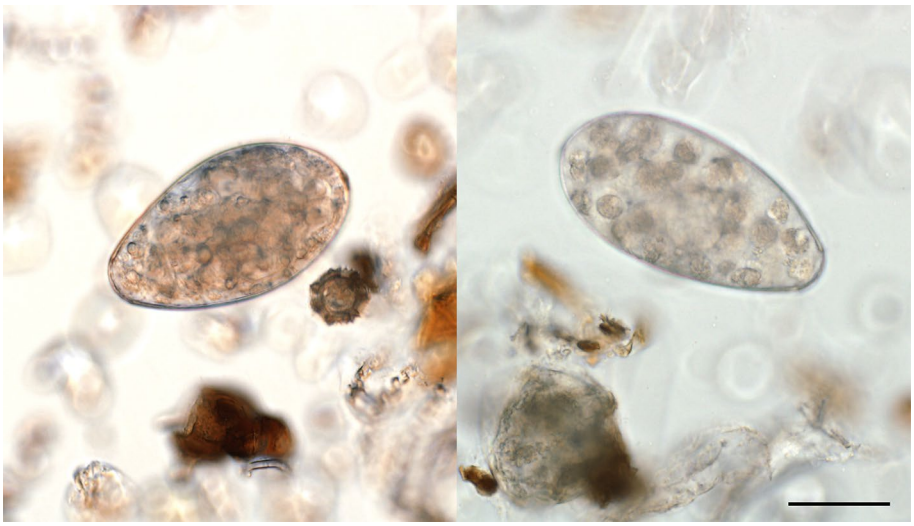
RESULTS

Between March 2019 and June 2021, total of 115 farms across the whole Czech Republic participated in the project, resulting into 5,573 beef cattle fecal samples sent to the laboratories of VETUNI and SVI. All but one of the Czech Republic regions were represented by 1 to 26 farms per region. Moravian-Silesian Region (T) had the highest number of participating farms, while South Moravian (B) and Central Bohemian (S) Regions were both represented by a single farm. The number of samples per region varied from 34 in South Moravian (B) Region to 1,299 in South Bohemian Region (C) (Fig. 1).

The results of coproscopic examination are summarized in Tab. I; 821 samples (14.7%) tested negative while 4,752 samples (85.3%) contained detectable stages of at least one parasite. The distribution of negative samples across the Czech Republic varied, fluctuating in a range 4.1–23.4%. The infections by a single parasite type were relatively frequent, including 818 samples with strongylid nematode eggs (14.7%), 756 samples with *Eimeria* only (13.6%), 450 samples with eggs of paramphistomid flukes (8.1%) and six samples (0.1%) with *Moniezia* only. Almost one third of



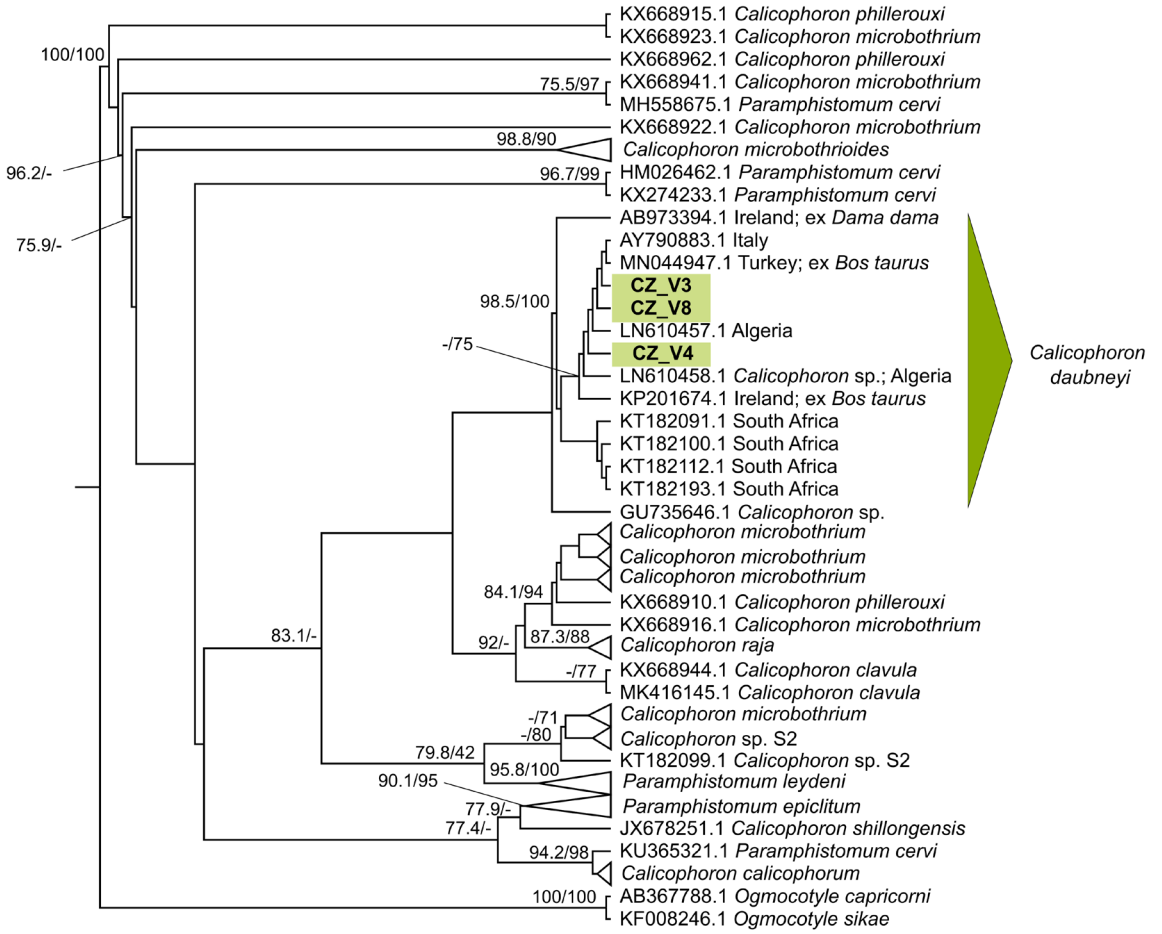
1: *Map of the Czech Republic Regions with the number of farms participating (left) and fecal samples collected (right) for each region. A – Praha, B – South Moravian, C – South Bohemian, E – Pardubice, H – Hradec Králové, J – Vysočina, K – Karlovy Vary, L – Liberec, M – Olomouc, P – Plzeň, S – Central Bohemian, T – Moravian-Silesian, U – Ústí nad Labem, Z – Zlín.*
Adjusted from: CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=912955



2: *Eggs of rumen flukes (identified based on ITS-2 sequences as Calicophoron daubneyi) detected by the sedimentation technique in a cattle sample originating from a beef farm in the South Bohemia region; scale bar = 50 μm*

samples (32.1%) showed co-infection by at least two of the three dominant taxa (strongylids, *Eimeria*, paramphistomids); the co-infection with all three dominant parasite taxa was identified in 8.4% of samples.

The eggs of paramphistomid flukes were identified in a total of 1668 fecal samples (29.9%) based on their morphology (Fig. 2). Randomly selected samples from three regions (C, K and U) were sequenced. All three ITS-2 sequences obtained were identical (uploaded to GenBank under accession number OK416067). BLAST search showed 100% identity to two previously published sequences of *Calicophoron daubneyi* from Ireland (KP201674.1) and Italy (AY790883) and to nine *C. daubneyi* sequences directly submitted to GenBank originating from Turkey (MN044947.1), Algeria (LN610457–8.1) and South Africa (KT182091–96.1). The final alignment for the maximum-likelihood phylogenetic tree comprised of 114 sequences, including two *Ogmocotyle capricorni* and *O. sikae* sequences as an outgroup. The length of the alignment was 452 nucleotides with 286 constant sites and 134 parsimony informative sites. The tree was calculated according to the K2P+G4 model. In the consensus tree, clades corresponding to species were formed, however, *Calicophoron phillerouxi*, *Calicophoron microbothrium* and *Paramphistomum cervi* sequences clustered in multiple clades (Fig. 3). All sequences of *Calicophoron daubneyi*, including



3: *Maximum-likelihood tree calculated by K2P+G4 model from a 452 bp alignment of 109 paramphistomid trematode ITS-2 region sequences downloaded from GenBank and three sequences obtained in this study (in green). Sequences of Ogmocotyle spp. were used as outgroup. Monophyletic clades corresponding to species are collapsed. Sequences are marked by the GenBank accession number. For C. daubneyi downloaded from GenBank, country of origin and host species are given when available. Numbers at the nodes are SH-like approximate likelihood/ultrafast bootstrap support in %. Only values above 75 are shown.*

our sequences, formed a separate clade closely related to other *Calicophoron* spp. and rather distant from *Paramphistomum* spp. clades. The sequences within the *C. daubneyi* clade originating from cattle, sheep and European fallow deer from Italy, Ireland, South Africa, Turkey and Czech Republic differed by a maximum of 0.8%.

DISCUSSION

Aiming at the helminth parasite spectrum in beef cattle, we examined more than 5,000 samples from 115 farms across the whole Czech Republic, providing the most extensive analysis of cattle parasites conducted so far in the country. Even though all but one regions of the Czech Republic were represented, the number of farms varied; the same way, also numbers of individual samples varied among farms, depending on farmers' attitude and logistics. However, the results in spectrum of detected parasites are homogeneous among the regions (Tab. I). This is not surprising, considering rather uniform climate and landscape through the country, common transportation of animals and uniformity of breeding and deworming practices.

The parasite spectrum of beef cattle in the Czech Republic is overall dominated by three taxa - strongylids, coccidia of the genus *Eimeria* and paramphistomids, which corresponds with previous studies conducted in the Czech Republic (e.g. Chroust *et al.*, 2006; Hromadiová, 2012; Kváč, 2003) as well as in Europe (e. g. Stancampiano *et al.*, 2007; Taylor, 2010; Theodoropoulos *et al.*, 2010). *Eimeria* infections are repeatedly listed (Chroust *et al.*, 1966, 2006; Koudela, 2007; Mottlová, 2008), with the highest prevalence and infection intensities in calves, however, age categories were not evaluated in presented study. Strongylid nematodes are the most common parasites found previously in the Czech cattle (Chroust *et al.*, 1982, 2006; Kubelka *et al.*, 2016), although in low infection intensities (Chroust, 2006; Kubelka *et al.*, 2016). Apparently, the situation remains relatively stable and our finding corresponds well with data from previous decades. So far, strongylid infections in beef cattle farms in the territory are not perceived

I: *Results of coproscopic diagnostics of parasites in beef cattle herds, divided by individual regions; only parasites with prevalence higher than 1% are shown, together with F. hepatica. A – Praha, B – South Moravian, C – South Bohemian, E – Pardubice, H – Hradec Králové, J – Vysočina, K – Karlovy Vary, L – Liberec, M – Olomouc, P – Plzeň, S – Central Bohemian, T – Moravian-Silesian, U – Ústí nad Labem, Z – Zlín.*

District			Strongylids	Paramphistomidae	Eimeria	Moniezia	Strongyloides	Trichuris	Fasciola
B	Farms	1	1	1	1	0	0	0	0
	Samples	34	24	1	23	0	0	0	0
			70.6%	3%	67.6%				
C	Farms	21	21	16	20	10	6	9	1
	Samples	1299	759	586	699	45	21	19	1
			58.4%	45.1%	53.8%	3.5%	1.6%	1.5%	0.07%
E	Farms	4	4	2	4	3	1	0	0
	Samples	123	56	20	44	6	1	0	0
			45.5%	16.3%	35.8%	4.9%	0.8%		
H	Farms	4	4	4	4	3	1	2	0
	Samples	357	182	10	179	11	1	8	0
			50.1%	2.8%	50.1%	3.1%	0.3%	2.2%	
J	Farms	5	5	1	5	3	2	0	0
	Samples	170	128	31	64	6	4	0	0
			75.3%	18.2%	37.6%	3.5%	2.4%		
K	Farms	7	7	6	7	2	3	2	3
	Samples	628	301	305	300	4	6	5	11
			47.9%	48.6%	47.8%	0.6%	1%	0.8%	1.8%
L	Farms	14	14	12	14	6	2	1	4
	Samples	765	417	233	350	17	2	1	11
			54.5%	30.5%	45.8%	2.2%	0.3%	0.1%	1.4%
M	Farms	4	4	3	4	3	1	1	0
	Samples	219	182	17	167	18	1	1	0
			83.1%	7.8%	76.3%	8.2%	0.5%	0.5%	
P	Farms	16	16	9	15	5	1	2	1
	Samples	457	227	124	221	8	5	3	1
			49.7%	27.1%	48.4%	1.8%	1.1%	0.7%	0.2%
S	Farms	1	1	0	1	0	0	0	0
	Samples	64	23	0	39	0	0	0	0
			35.9%		60.1%				
T	Farms	26	25	14	25	8	3	5	3
	Samples	783	391	227	443	13	5	9	5
			49.9%	29%	56.6%	1.7%	0.6%	1.1%	0.6%
U	Farms	8	8	3	8	3	3	4	0
	Samples	184	137	49	98	3	7	7	0
			74.5%	26.6%	53.3%	1.6%	3.8%	3.8%	
Z	Farms	4	4	3	4	2	3	1	0
	Samples	490	323	65	303	5	12	1	0
			65.9%	13.3%	61.8%	1%	2.4%	0.2%	
Total	Farms	115	114	74	112	48	26	27	12
	Samples	5573	3150	1668	2930	136	65	54	29
			56.5%	29.9%	52.6%	2.4%	1.2%	1%	0.5%

as a serious issue (personal communication with farmers). Strongylid nematodes in general can pose serious health problems (Fox and Jacobs, 1981) followed by economic loses (Corwin, 1997) and hand in hand with increase of populations of strongylids resistant to the most commonly used anthelmintics (Sutherland and Leathwickthey, 2011) they pose rapidly escalating problem in cattle. Concentration coproscopic diagnostics followed by microscopy does not allow identification of strongylids even at the genus level. Thus, more sophisticated methods are to be applied to reveal real spectrum of strongylid nematodes involved in infections (Avramenko *et al*., 2015).

The dominant finding of the study was detection of eggs of paramphistomid rumen flukes observed in almost 30% of samples examined. However, their presence (at least one positive sample from given farm) was detected in 64% farms in all regions except for the Central Bohemian Region. In Karlovy Vary (K) and South Bohemian (C) regions, the percentage of the positive samples reached almost 50%. Barcoding of the ITS-2 region confirmed the identity of the detected paramphistomids as *C. daubneyi* (Dinnik, 1962). The three sequences from three different regions of the Czech Republic were identical. Similarly, a recent study (Sargison *et al*., 2019) showed low diversity of *C. daubneyi* ITS-2 across the United Kingdom, when only two haplotypes differing in a single nucleotide were detected among 32 parasite populations. Our ITS-2 sequences were identical to those previously published from Italy, Ireland, Turkey, Algeria and South Africa, demonstrating a low diversity of this paramphistomid fluke in Europe and suggesting recent invasion from limited source. Even though cattle represent a dominant host of *C. daubneyi* in Europe, the spectrum of reported hosts comprises also other domestic (sheep, zebu, water buffalo) and free-ranging ruminants (mouflon, various deer), recently extended by llama and alpaca infected by *C. daubneyi* (Eduardo, 1983; Mitchell *et al*., 2021). Apparently, broad occurrence in Czech beef cattle herds supports a risk of spillover to other ruminants

Paramphistomosis in ruminants caused by *C. daubneyi* has emerging character in Europe (Atcheson *et al*., 2020; Huson *et al*., 2017) and even though the infection is usually subclinical, weight loss, anorexia, depression, dehydration, diarrhea, enteritis, and mesenteric lymphadenopathy may be observed, usually linked to early infection stage when immature flukes are present (Chroust, 1964; Malrait *et al*., 2015). In some of the herds, farmers reported presence of low numbers of animals with clinical signs attributable to clinical paramphistomosis, however, the clinical signs are so unspecific, that it is rather premature to draw any conclusions about the impact of the parasite on overall health of beef cattle (Atcheson *et al*., 2020).

Parasitological studies on herbivores performed between 1960's to 1980's in the Czech Republic reported occurrence of *Paramphistomum ichikawai* and *P. cervi* in the cattle herds (Chroust, 1964; Kotrlá and Chroust, 1978); same flukes were commonly observed also in game animals (Kotrlá and Kotrlý, 1982). However, there were no reports of paramphistomid flukes in past decades and the broad occurrence of rumen flukes in the examined cattle in our study demonstrate substantial shift. As the flukes in the three randomly selected farms from various regions of the country were invariably identified as *C. daubneyi,* we assume that most (if not all) findings of rumen fluke eggs in cattle are attributable to this species, making *C. daubneyi* one of the most frequent helminths in beef cattle herds. Similar shift in the occurrence of paramphistomid flukes was observed in UK and Ireland where only *Paramphistomum* spp. were observed in cattle during the 1950's (Willmott, 1950), being replaced by *C. daubneyi* which is currently the dominant species (Mitchell *et al*., 2021; Zintl *et al*., 2014). Considering broad distribution of *C. daubneyi* in Western Europe (Huson *et al*., 2017) and extensive beef cattle importations to the Czech Republic in past decades, it is probable that parasite was recently imported to the Czech herds and consequently spread across the whole territory of the Czech Republic.

Described shift in spectrum of paramphistomids of cattle has also range of ecological connotations. European species of *Paramphistomum* typically exploit planorbid aquatic snails as their intermediate hosts. On the contrary, the dominant intermediate host of *C. daubneyi* is *Galba truncatula* (and few other lymnaeid snails) (Atcheson *et al*., 2020; Huson *et al*., 2017). The beef cattle production in the Czech Republic has moved to areas with higher altitude with conditions perfect for *G. truncatula*, but not for most of the planorbids. Importantly, the same spectrum of mollusks is involved in the life cycle of a liver fluke *Fasciola hepatica* (Abrous *et al*., 2000; Beesley *et al*., 2017; Deplazes *et al*., 2016). Compared to *C. daubneyi*, *F. hepatica* is less prevalent in the Czech Republic (Zmuda and Chroust, 2001) and also in our study, the liver fluke was detected in less than 30 samples at 12 farms in five regions. Fasciolosis was causing quite big economic loss in the past, however, extensive use of anthelmintic treatment apparently reduced the liver fluke populations while *C. daubneyi* is resistant to most of the anthelmintics used in management of fasciolosis. The common occurrence of *C. daubneyi* in Czech farms indicates environmental conditions suitable also for *F. hepatica* and risk of its emergence.

Presented data show the necessity of continuing of active surveillance of helminths in beef cattle in the Czech Republic, targeting not only the overall spectrum of detected helminths, but also composition of communities of strongylid

nematodes and correct identification of rumen flukes. None of the farms investigated in our study reported significant problems with helminthiases, which can be caused by lack of awareness rather than by absence of clinical infections. Reported presence of rumen flukes apparently deserves more attention not only from the state veterinary service but also on side of individual farmers and veterinarians in the field.

CONCLUSION

We examined more than 5,000 fecal samples from beef cattle farms in Czechia using routine coproscopic methods. Beside the infection by gastrointestinal strongylid nematodes and coccidia, *Calicophoron daubneyi* was detected in more than 60% of examined farms. The ITS-2 DNA barcoding confirmed the identity of the detected paramphistomids and genetic uniformity with European isolates of this invasive fluke.

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