EIMERIA AND *CRYPTOSPORIDIUM* IN LITHUANIAN CATTLE FARMS

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Summary. Infections with *Eimeria* and *Cryptosporidium* in cattle are globally prevalent. However, little is known on the prevalence and species of these infections in Lithuania. The objective of the study was to determine the levels of infection of coccidia at 7 Lithuanian cattle farms. We aimed at establishing an estimate of prevalences of animals shedding coccidia oocysts and species as well as infection intensities from different age categories. Quantitative flotation of 15 faeces samples from each farm, stratified on cattle <3, 3-12, and >12 months of age, were investigated for *Eimeria* with the modified McMaster technique. *Cryptosporidium* samples were investigated using acid fast contrast staining (Ziehl-Neelsen) and given a semi-quantitative oocyst count. *Eimeria* oocysts had been sporulated and morphologically differentiated. All farms had both coccidia. *Cryptosporidium* was evenly distributed in the different age groups, while *Eimeria* was found mainly in animals >3 months. Oocyst counts were generally low for both pathogens. Eleven species *of Eimeria* were identified, mostly pathogenic species. Coccidia are heavily integrated in Lithuanian cattle farms in all ages and call for more attention.
Key words: coccidia, *Cryptosporidium, Eimeria,* cattle, Lithuania.

EIMERIA IR CRYPTOSPORIDIUM INVAZIJOS PAPLITIMAS LIETUVOS GALVIJŲ FERMOSE

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Santrauka. *Eimeria* ir *Cryptosporidium* kokcidijos yra plačiai paplitusios ir sukelia galvijų sveikatos problemų visame pasaulyje. Kadangi apie šių parazitų atskirų rūšių paplitimą tarp Lietuvos galvijų literatūroje duomenų rasta nedaug, buvo atliktas tyrimas, kurio metu eimeriozės ir kriptosporidiozės ištirtos septyniose galvijų fermose. Tirta atskirų pirmuonių rūšių paplitimas ir invazijos intensyvumas pagal gyvulio amžių. Flotacijos metodu ištirta po 15 išmatų mėginių iš kiekvienos fermos, galvijai suskirstyti į grupes pagal amžių: jaunesni kaip 3, 3–12 ir vyresni nei 12 mėnesių. Eimeriozė buvo tiriama MacMaster metodu, o kriptosporidijų oocistų buvo imami tepinėliai, dažomi Ziehl–Neelsen metodu, ir tada skaičiuojama mikroskopu. *Eimeria* oocistos buvo sporuliuojamos ir diferencijuojamos pagal rūšis. Visose tirtose fermose rasta ir eimerijų, ir kriptosporidijų. *Cryptosporidium* buvo tolygiai paplitusios įvairaus amžiaus grupėse, tuo tarpu *Eimeria* dažniausiai rasta vyresnių nei 3 mėn. galvijų organizme. Vidutinis oocistų kiekis 1 grame išmatų buvo nedidelis visose gyvulių grupėse. Nustatyta 11 *Eimeria* patogeninių rūšių. Mūsų tyrimų rezultatai parodė, kad eimerijos ir kriptosporidijos Lietuvos fermose yra plačiai paplitusios tarp įvairaus amžiaus grupių galvijų.
Raktažodžiai: kokcidijos, *Cryptosporidium, Eimeria,* galvijai.

Introduction. Coccidia are one of the most common intestinal parasites of large animals and are a cause of disease and production losses for animals in captivity (Fitzgerald, 1980; Burger, 1983). Most important coccidia in cattle are *Eimeria* spp. and *Cryptosporidium* spp. Clinical symptoms often mainfest as heavy watery diarrhoea. The signs may however be moderate or absent even in severe infections (Cornelissen et al., 1995). Commonly milder symptoms are overlooked, and farmers and veterinarians are not aware or uncertain how to address the problems of coccida, resulting in life long consequenses to animal health (Fitzgerald, 1980; Fox, 1985). Naturally infected calves may fall behind healthy siblings with 110-270 grams per day the first months of life, and the lost weight does not seem to be regained with time (Fitzgerald, 1980; Nielsen et al., 2003; Samson-Himmelstjern et al., 2006). First step in dealing with coccidial problems is to unveil their presence to the animal tenders and investigate the extent of the spread. Species differentiation is important to determine the severity and nature of the infections (Daugschies, Najdrowski, 2005). To our knowledge little or nothing is published of the current status of coccidia in the Baltic countries. This study is part of a larger investigation of cattle coccidia in Estonia. The objective of the study was to estimate the spread of coccidia in Lithuanian cattle farms for more complete picture of the Baltic situation.

Materials and Methods. Population and sample collection.

Samples from 7 volunteering Lithuanian cattle farms were collected in May 2007. Each farm provided 15 samples, evenly from three different age groups: <3, 3-12, and >12 months. The samples were collected from rectum into disposable gloves and kept cooled prior investigation.

Flotation and concentration McMaster.

The consistency of faeces were categorized as: hard and dry, normal, soft, thin, watery, watery and bloody. The last two categories were classified as "diarrhoeic". Samples were analysed with the concentration McMaster technique described by Roepstorff and Nansen (1998). Following dissolving, filtering, and centrifuging 4 grams faeces, the pellet was dissolved in a saturated sugar and salt solution (p=l.26 g/cm^3) shortly before analysis. Counting chambers were specially constructed of 76x26mm microscopic slides and a 0.1 mm cover slide (Knitel-Glaser) (Henriksen, Korsholm, 1984). The amount of oocysts present in one gram of faeces (OPG) were counted for each sample and classified as low (50-1,000), medium (1,001-5,000), and high (>5,000). To test for false positives 1-2 water samples were processed with each herd samples.

Sporulation *of Eimeria* and species differentiation.

Samples with *Eimeria* oocysts were sporulated using 2.5% w/v potassium dichromate ($K_2Cr_2O_6$, Riedel-de Haen), and aired by using a pipette and left at room temperature up to 3 weeks before investigation. The whole chamber was searched to find as many different species as possible. When enough oocysts were available 30 oocysts were studied in detail. Oocysts were measured using an eyepiece micrometer (Ceti) and the species determined by size, shape, colour, and morphological appearance (Levine, 1985). The species found were classified as: highly pathogenic (HP, *E. bovis* and *E. zuernii),* low pathogenic (LP, *E. ellipsoidalis, E. alabamensis, E. auburnensis, E. subspherica),* and non-pathogenic (NP) (Ernst, Benz, 1986; Cornelissen et al., 1995; Autzen et al., 2002).

Cryptosporidium contrast staining and differentiation.

Thin smears of faeces were applied on microscope slides, dried at room temperature, and stained with a modified Ziehl-Neelsen technique (Henriksen, Pohlenz 1981). Briefly, the smears were fixed 2-5 minutes in a methanol with 10% (v/v) hydrochloric acid (36% HC1, P. Ch. "Stanchem", Poland). The dried slides then spent 20 minutes in Carbolic Fuxine solution (Carl-Roth, Germany), removing of excess colour by 2 repeats of 10 second in 10% (v/v) sulphuric acid (96% H_2SO_4, Lach-Ner, s.r.o, Czech Republic) and rinsing in tap water. Finally, slides spent 5 minutes in Malachite green G solution (Carl-Roth, Germany), washed in tap water, and air dried. Positive controls had previously been established and were included in all stainings. All positive samples were scored semi-quantitatively from the average number of oocysts per visual area (ova): 1−5 ova = 10^4-10^5, 6-25 ova = 10^5-10^6, and >25 ova > 10^6.

Results. All herds (100%) investigated had animals shedding both *Eimeria* spp. and *Cryptosporidium* spp. (Table 1). *Eimeria* was mainly found in animals older than 3 months, whereas *Cryptosporidium* was slightly more frequent in animals younger than one year. The sample frequency *of Eimeria* species found in positive herds and samples are shown in Table 2. On average 7 different species were found in the herds. Distribution of non-pathogenic, low pathogenic, and highly pathogenic species are presented in Figure 1. The number of different *Eimeria* species found in samples is presented in Table 3. Only four animals scored as diarrhoeic, all from different farms, evenly devided between animals of <3 and >12 months of age. All the diarrhoeic animals had low infections *of Cryptosporidium* spp. but only one shed *Eimeria* spp. oocysts (OPG=183). The distribution of low, medium, and high oocyst counts is presented in Table 4.

Table 1. **Sample and herd prevalences of *Eimeria* spp. and *Cryptosporldlum* spp. in cattle farms**

Farm	Coccidia	Herd prevalence	Sample prevalence (%)			OPG counts
			<3 months	3-12 months	>12 months	
1	*Eimeria* spp.	73	80	100	40	0-3892
	Cryptosporidium spp.	80	60	100	80	0-10^4
2	*Eimeria* spp.	67	20	100	80	0-2780
	Cryptosporidium spp.	20	20	0	40	0-10^4
3	*Eimeria* spp.	67	0	100	100	0-834
	Cryptosporidium spp.	47	100	20	20	0-10^4
4	*Eimeria* spp.	73	20	100	60	0-9730
	Cryptosporidium spp.	93	80	100	100	0-10^6
5	*Eimeria* spp.	53	40	80	40	0-1390
	Cryptosporidium spp.	80	80	100	60	0-10^6
6	*Eimeria* spp.	27	0	20	60	0-16860
	Cryptosporidium spp.	67	80	80	40	0-1 0^s
7	*Eimeria* spp.	27	0	40	40	0-556
	Cryptosporidium spp.	80	60	80	100	0-10^6
Average;	*Eimeria* spp.	55; 27-73	23; 0-80	77; 20-100	60; 40-100	604; 0-16860
range	*Cryptosporidium* spp.	67; 20-93	69; 20- 100	69;0-100	63; 20-100	10^4;0-10^6
OPG = oocysts / 1 gram faeces						

Table 2. **Distribution of bovine *Eimeria* species in herds and positive samples**

Species	Herds		Positive samples	
	N	%	N	%
E. alabamensis	6	86	16	27
E. auburnensis	5	71	19	32
E. bovis	7	100	30	51
E. brasiliensis	1	14	2	3
E. bukidnonensis	0	0	0	0
E. canadensis	5	71	16	27
E. cylindrica	1	14	5	8
E. ellipsoidalis	7	100	15	25
E. pellita	1	14	2	3
E. subspherica	3	43	4	7
E. wyomingensis	3	43	2	3
E. zuernii	5	71	25	42

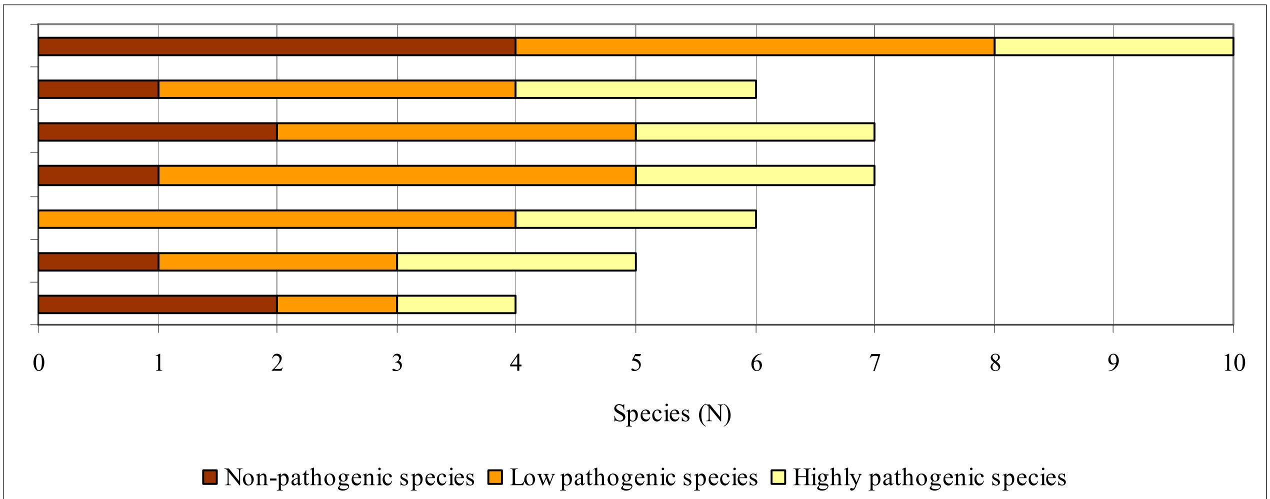

Figure I. **Distribution of non-pathogenic** *(E. brasiliensis, E. bukidnonensis, E. canadensis, E. cylindrica, E. pellita, E. wyomingensis),* **low pathogenic** *(E. alabamensis, E. auburnensis, E. ellipsoidalis, E. suspherica),* **and highly pathogenic** *(E. bovis* and *E. zuernii)* ***Eimeria* species in cattle farms**

Table 3. **Number of *Eimeria* species found in cattle herds**

	Number of species in samples (N)							
	0	1	2	3	4	5	6	7
Percentage (%)	45	23	8	13	9	2	0	1

Table 4. **Distribution** (%) **of oocyst counts** (oocysts per gram faeces, OPG)

Age	Coccidia	Low[a]	Medium[a]	High[a]
<3 months	*Eimeria* spp.	70	20	10
	Cryptosporidium spp.	83	13	4
3-12 months	*Eimeria* spp.	78	18	4
	Cryptosporidium spp.	74	13	13
> 1 2 months	*Eimeria* spp.	86	14	0
	Cryptosporidium spp.	92	4	4
All	*Eimeria* spp.	78	17	5
	Cryptosporidium spp.	83	10	7

[a]Low, medium and high OPG is defined respectively for *Eimeria* as: 50-1000, 1001-5000, >5000, and for *Cryptosporidium:* 10^4-10^5, 10^5- 10^6, $>10^6$

Discussion. All Lithuanian farms investigated had animals with both *Eimeria* spp. and *Cryptosporidium* spp. The high national prevalence is shared with close neighbours such as Poland and Estonia (Klockiewicz et al., 2007; Lassen et al., 2009). It is possible that the small sample size and the narrow time window of sampling gives a screwd representation of the situation in Lithuania. Average herd prevalences for the studied farms are 3 times higher for *Cryptosporidium* than in Estonia, and 15% higher for *Eimeria* (Lassen et al., 2009). Similarly, the age group repensentation *of Cryptosporidium* in samples is about twice that found in Estonia. The distribution of the positive *Cryptosporidium* samples in the different age categories is similar in the two countries, being almost uniform. This does not follow the general knowledge that older cattle would not be infected so often due to acquired immunity (Maddox-Hyttel et al., 2006). *Eimeria* infections in calves <12 months seems to be fairly similar to Estonia, but very high for older animals in Lithuania. Calves between 3-12 months of age were most commonly infected with *Eimeria,* and a lot more than compared to younger calves. Animals in the range of 3-6 months have been noted as more susceptible to this infection (Taylor, Catchpole, 1994). As was seen for *Cryptosporidium* older animals shed *Eimeria* oocysts frequently, and at low levels. If the environment is heavily contaminated it may explain the findings by a frequent uptake of oocysts resulting in low excretion from adult animals that would normally have developed some immunity. Constant reinfections as supposed for *Eimeria* may very well be the case for *Cryptosporidium* as well. There was little difference in infection intensities between age categories, but low oocyst counts of either coccidia was more commonly seen in cattle >12 months, while higher counts were observed in calves <12 months of age. Of the most common bovine *Eimeria* species 11 were identified. An average of 7 species per farm were found: 0-4 non-pathogenic, 1-4 low pathogenic, while the pathogenic *E. ziiernii* and *E. bovis* were found in almost all herds. *Eimeria bukidnonensis* was the only species not observed. The high frequency of rarer species such as *E. brasiliensis, E. pellita,* and *E. wyomingensis,* was unexpected considering the modest sample size. It may indicate these species are not so uncommon in Lithuania. Distribution of low pathogenic species were quite similar to other studies in Nothern Europe (Autzen et al., 2002; Klockiewicz et al., 2007; Stewart et al., 2008), but less than what is observed in Estonia. For the remaining species this study looks like findings from organic Danish cattle herds (Vaarst et al., 2003) with the exception that *E. cylindrica* is a less common in Lithuanian herds. Too few cases of diarrhoea was observed for any statistical investigation of relationships to the oocysts counts or infected animals with coccidia, and was only found in cattle <3 and >12 months of age.

Conclusions. Coccidia were found in all farms, and commonplace even in older animals, perhaps as passants or low level re-infections. A high variety *of Eimeria* species were identified in the investigated farms, potentially exposing the animals to many infections throughout its life time. Diarrhoeic samples were uncommon. Potential for coccidial problems exist for cattle in Lithuania, and calls for further investigations and to create awareness.

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