THE PREVALENCES OF *EIMERIA* AND *CRYPTOSPORIDIUM* IN LARGE LATVIAN CATTLE HERDS

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Summary. *Eimeria* and *Cryptosporidium* are common intestinal pathogens known to potentially cause long term effects to cattle health and production. This study aimed to document these parasites in Latvian cattle herds. From 16 dairy farms, 125 faecal samples were collected from animals aged 8–20 weeks. In addition, seven farmers completed a questionnaire aimed to assess their general knowledge on parasites, including questions regarding parasites and usage of anthelmintics, as well as grazing and housing practices, and occurrence of diarrhoea in calves. The faecal samples were examined for the presence of *Eimeria* oocysts using a McMaster technique and for the presence of *Cryptosporidium* oocysts with contrast staining of faecal smears. *Eimeria* species were differentiated morphologically. All herds were positive for *Eimeria* spp. and *Cryptosporidium* spp. was detected in 69% (95% CI 58-80) of them. *Eimeria* was found in 46% (95% CI 30-62) of individual samples with mean oocyst count of 7935 (SD 18022) per gram of faeces. Eight species were identified; *E. zuernii* and *E. bovis* were the predominant ones. *Cryptosporidium* was detected in 41% (95% CI 34-48) of examined animals but predominantly at low infection levels. Farmers, however, did not consider parasites as a problem. Combined low awareness among farmers, high infection pressure with *Eimeria* indicated by high oocyst counts of pathogenic species in most cases, and high herd-level prevalence of *Cryptosporidium* suggests that these parasites may be overlooked.

Keywords: *Eimeria*, *Cryptosporidium*, cattle, calf, diarrhoea, Latvia.

PARAZITINIŲ PIRMUONIŲ *EIMERIA* IR *CRYPTOSPORIDIUM* PAPLITIMAS LATVIJOS GALVIJŲ BANDOSE

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Santrauka. Nustatyta, kad *Eimeria* ir *Cryptosporidium* yra paplitę virškinamojo trakto parazitai, turintys įtakos galvijų sveikatingumui ir produktyvumui. Atliktas tyrimas, norint nustatyti šių parazitų paplitimą Latvijos galvijų bandose. Iš 16 galvijų fermų paimti 8–20 savaičių 125 galvijų fekalijų mėginiai. Be to, septyni ūkininkai užpildė klausimyną, kur atsakė į bendrus klausimus apie parazitus, taip pat apie antihelmintikų panaudojimą, galvijų ganymą, laikymą, veršelių diarėją. Tyrimai atlikti McMaster metodu (eimerijų oocistų) ir kontrastiniu dažymo metodu (kriptosporidijų oocistų). *Eimeria* rūšys buvo identifikuotos morfologiškai. Mūsų tyrimai parodė, kad visos galvijų bandos buvo teigiamos *Eimeria* spp. ir *Cryptosporidium* spp. atžvilgiu. Rasta 69 proc. teigiamų mėginių (95 proc. CI 58–80). *Eimeria* rasta 46 proc. (95 proc. CI 30-62) mėginių esant vidutiniškai 7935 oocistoms (SN 18022) grame fekalijų. Aštuonios eimerijų rūšys buvo identifikuotos, o *E. zuernii* ir *E. bovis* buvo vyraujančios. *Cryptosporidium* dažniausiai žemu invazijos lygiu buvo rastos 41 proc. tirtų mėginių (95 proc. CI 34–48). Ūkininkų apklausa parodė, kad parazitų invazija nelaikoma rimta problema, o žinios apie parazitus ir su jais susiję klausimai buvo minimalūs. Ištirta, kad parazitiniai pirmuonys yra ženkliai paplitę Latvijos galvijų bandose, tačiau ūkininkai nėra informuoti nei apie šiuos parazitus, nei apie jų keliamas problemas bei patologijas. Taigi reikalingi tolimesni tyrimai, o ypač ūkininkų švietimas šiais klausimais.

Raktažodžiai: *Eimeria*, *Cryptosporidium*, galvijai, veršeliai, viduriavimas, Latvija.

Introduction. *Cryptosporidium* and *Eimeria* are among the most common and important enteropathogens in calves (Cornelissen et al., 1995; Maddox-Hyttel et al., 2006; Bartles et al., 2010). The most important clinical sign they may cause is thin watery diarrhoea, occasionally containing blood in *Eimeria* infections. Other less obvious signs are fatigue, fever, and reduced appetite (Fitzgerald, 1980). High infection pressure, and lack of preventive and therapeutic measures when clinical disease manifests, can lead to outbreaks and high mortality among calves (Fox, 1985). Moreover, the sub-clinical effects of infection, which often result in recovery but lower weight gain, are also important to animal health and productivity (Daugschies et al., 1986).

Recent studies have shown that *Eimeria* and *Cryptosporidium* are widespread in Lithuanian and Estonian cattle herds, but these parasites appear to be of low priority when questioning the farmers and observing statistics of veterinary diagnostics (Lassen, 2009; Lassen and Järvis, 2009). However, too little information on the prevalence and importance of these parasites in Latvian cattle herds is published.

The aim of this study was therefore to examine the herd and individual sample level prevalences of *Eimeria* and *Cryptosporidium* in large Latvian cattle herds. In addition to oocyst counts, the *Eimeria* were identified to the species level to reveal the distribution of pathogenic and non-pathogenic species in the samples. The presence of diarrhoea was also recorded. To acquire a general impression on the farmers' attitude towards parasite related problems, a questionnaire was administered to farmers.

Materials and Methods

Population and sampling

The required sample size was calculated with Survey Toolbox 1.0 (AusVet Animal Health Services, 1996) using 79% sensitivity, 93% specificity, population size of 200 calves, and expected minimal prevalence of 20% (Quilez et al., 1996). Sixteen voluntarily participating dairy farms were included in the study. The size of the sampled herds varied between 150-1200 animals (mean 500, SD: 419), and they constitute the 11% of Latvian herds with more than 100 animals (Berke, 1997). The herds were situated in four of five Latvias' regions: Kurzeme (4), Latgale (3), Riga (5) and Zemgale (4); in counties Dobeles (3), Riga (1), Kokneses (1), Ogres (1), Tukuma (3), Saldus (3), Rezeknes (2), Vilanu (1) and Talsu (1).

The farmers were asked to collect the number of individual faecal samples that was calculated according to how many calves they had. Following this instruction, individual samples (6-26 samples per farm) were collected and delivered from 9 farms. From 7 farms, pooled samples (each from 5-10 animals) were received.

The samples were transported in a cooler and stored at +4^{o}C until examined. Altogether 125 faecal samples (11 pooled samples and 114 individual samples) from housed calves ranging from 8 to 20 weeks of age were examined in March and September 2009.

Quantitative flotation

Faeces were categorised as diarrhoeic if liquid in consistency at room temperature. The investigation was performed by a concentration McMaster technique, as described by Roepstorff and Nansen (1998), using a flotation fluid of 500 g/L sugar saturated with salt (r = 1.30 g/cm3). The counting chambers were constructed of microscopic slide (76 x 26 mm) and a 0.1 mm cover slide (Knitel-Gläser), as described by Henriksen and Korsholm (1984). The total volume of the faecal suspension in the chamber was 0.6 ml of which the area of 3 vertical rows (0.07 ml) was observed and oocysts were counted at X 200 magnification.

Identification of *Eimeria* to species level

The *Eimeria* oocysts found were sporulated using 2.5% w/v potassium dichromate ($K_2Cr_2O_6$, Riedel-de Häen), frequent aeration with a pipette, and incubation at room temperature for up to 3 weeks before examination. Each sample was examined with a light microscope at X 400 magnification for morphological identification of *Eimeria* to species level. The species were determined by size, shape, colour, and morphological appearance of oocysts (Levine, 1985). The dominant species was determined by identifying 10% of the total OPG (oocysts per gram of faeces) count of the sample, but examining at least 10 oocysts per sample.

Contrast staining for *Cryptosporidium* oocysts

Thin smears of faeces were applied on microscope slides, air dried, and stained with a modified Ziehl–Neelsen technique, as described by Henriksen and Pohlenz (1981). Positive controls were included in all stainings. Each entire sample (approximately 24x32 mm) was examined at X 400 magnification to detect *Cryptosporidium* oocysts. In positive samples, oocysts were counted in three randomly selected view fields at X 400 magnification, and the mean was calculated. The positive samples were scored semi-quantitatively according to the mean count of oocysts per view area (OVA): 1–5 OVA (low), 6–25 OVA (medium), and >25 OVA (high).

Questionnaire data

Seven of the 16 questionnaires administered to the farmers were correctly filled for the analysis. The questions asked and options available were as follows: "Have you ever observed parasite problems in your farm?": Yes/No; "If yes, what parasites?"; "If yes, what treatment was administered to animals?"; "How often do you observe diarrhoea in calves younger than 2 months of age?": Never / Rarely / Occasionally / Always; "Do you consider diarrhoea in calves a problem in your herd?": Yes/No; "Are the calves pastured in spring?": Yes/No; "If yes, do you observe diarrhoea in calves grazing for the first time in the first weeks after turnout?": Yes/No; "When indoors, are the calves reared in a separate building from the older cattle?": Yes/No.

Data analysis

Pooled samples were only used to determine the prevalence of the protozoans in the herd level. The bovine *Eimeria* species were ranked in three groups as follows: pathogenic (*E. bovis* and *E. zuernii*), low-pathogenic (*E. ellipsoidalis*, *E. alabamensis*, *E. auburnensis*, *E. subspherica*), and non-pathogenic (Ernst and Benz, 1986; Cornelissen et al., 1995; Autzen et al., 2002). The animals examined were grouped into four age groups: 8 weeks of age or younger, 9-12, 13-16, and 17-20 weeks of age.

The *Cryptosporidium* semi-quantitative OVA-count had a Poisson distribution (mean / variation = 0.93) and were analysed accordingly. Differences between outcome variables (age and herd size) and dependent variables (*Eimeria* OPG counts, *Cryptosporidium* semi-quantitative scores, and number of *Eimeria* or *Cryptosporidium* positive animals) was analysed using linear regression analysis. The same approach was used to examine diarrhoea as dependent variable for OPGs and *Cryptosporidium* scores as outcome variables. Statistical calculations and analysis were performed with R statistics version 2.8.0 (The R Foundation for Statistical Computing).

Results

Eimeria

All herds were positive for *Eimeria* spp. Within the herds, *Eimeria* prevalences ranged 13-75% with a mean of 46% (95% CI 30-62), and mean oocyst count of 7935 OPG (SD: 18022). Although eight species were identified, mainly pathogenic, the diversity was low (Table 1 and Table 2). The prevalence was high among animals between 8-12 weeks of age, and oocysts were detected in half of the older animals but in lower numbers (Table 3). Individual sample prevalence reached 46% (95% CI 41-51) with a mean OPG count of 13765 (SD: 74282). Strongylids were the only other parasites found by flotation, detected in 7% (95% CI 5-9) of the samples.

Table 1. ***Eimeria* species in 16 large Latvian cattle herds, and in the 53 positive samples of 114 individual samples from 9 of the herds.** ALA = *E. alabamensis,* AUB = *E. auburnensis,* CAM = *E. canadensis,* CYL = *E. cylindrica,* ELL = *E. ellipsoidalis,* SUB = *E. subspherica,* BOV = *E. bovis,* ZUE = *E. zuernii*

	Low- and non-pathogenic species						Pathogenic species	
Species	ALA	AUB	CAM	CYL	ELL	SUB	BOV	ZUE
Herd prevalences (N=16)								
Positive % (95% CI)	3 19 (9-28)	3 19(9-28)	6 38 (26-49)	4 25 (14-36)	12 75 (64-86)	6 38 (26-49)	11 69 (57-80)	12 75 (64-86)
Presence in positive samples (N=53)								
Positive % (95% CI) mean OPG, (±SD)	9 17 (12-22) 826 (544)	2 4 (1-6) 1488 (1447)	3 6 (3-9) 992 (430)	4 8 (4-11) 6500 (4727)	19 36 (29-42) 7503 (13450)	7 13 (9-18) 8332 (14406)	24 45 (39-52) 28178 (109854)	39 74 (68-79) 86437 (18206)
Samples with species dominating (N=53)								
Positive % (95% CI) mean OPG, SD	5 9 (5-13) 780, 677	0 0 (-) -, -	1 2 (0-4) 1488, -	0 0 (-) -, -	5 9 (5-13) 855, 688	2 3 (1-6) 5468, 7601	17 32 (26-38) 4419, 9572	23 43 (37-50) 27557, 112326

CI = confidence interval, OPG = oocysts per gram of faeces, SD = standard deviation

Table 2. **Distribution of *Eimeria* and degrees of pathogenicity from 114 samples collected from large Latvian cattle herds**

N species per sample		0	1	2	3	4	5
All species (N=114)	N % (95% CI)	61 54 (49-58)	20 18 (14-21)	19 17 (13-20)	9 8 (5-10)	3 3 (1-4)	2 2 (1-3)
Low-pathogenic species (N=114)	N % (95% CI)	82 72 (68-76)	27 24 (20-28)	5 6 (3-6)	0 0	0 0	- -
Pathogenic species (N=114)	N % (95% CI)	68 60 (55-64)	29 25 (21-29)	17 15 (12-18)	- -	- -	- -

Low-pathogenic species: *E. alabamensis, E. auburnensis, E. ellipsoidalis, E. suspherica.* Pathogenic species: *E. zuernii* and *E. bovis*

Cryptosporidium spp.

Cryptosporidium spp. was found in 11 (69%; 95% CI 58-80) of the 16 farms. Oocysts were mainly found in animals of 8 weeks or between 12-13 weeks of age (Table 3). The stainings were positive in 41% (95% CI 34-48) of the samples and the distribution of semi-quantitative results of low, medium, and high scores were: 78% (95% CI 72-84), 15% (95% CI 10-20), and 9% (95% CI 5-13), respectively. The larger herds had more often lower semi-quantitative scores of *Cryptosporidium* in their samples (P<0.05) and tended to have fewer infected animals (P=0.06).

Table 3. The **prevalences of *Eimeria* and *Cryptosporidium* in calves from large Latvian cattle herds, by age group**

Age groups		≤8 weeks	9-12 weeks	13-16 weeks	17-20 weeks
Eimeria spp.	Positive / N Prevalence % (95 % CI) Mean OPG counts (± SD)	6 / 8 75 (60-90) 17486 (20966)	4 / 6 67 (48-86) 136744 (269362)	5 / 12 42 (28-56) 4523 (3900)	20 / 35 57 (49-65) 813 (823)
Cryptosporidium spp.	Positive/N Prevalence % (95 % CI)	4/8 50 (33-67)	2/6 33 (15-52)	8/12 67 (53-80)	12/35 34 (26-42)

CI = confidence interval, OPG = oocysts per fram of faeces, SD = standard deviation.

Diarrhoea

Diarrhoea was not common in the samples, and even less common in faeces with high OPGs and pathogenic *Eimeria* species (Table 4). Higher *Cryptosporidium* OVA-count (P=0.001) and presence of oocysts in samples (P=0.04) were associated with the presence of diarrhoea, but similar association between *Eimeria* and diarrhoea was not found.

Table 4. **Diarrhoea observed in faecal samples from Latvian calves** (N=114) **in regard: low OPG** (oocysts per gram faeces) **or higher, presence of pathogenic *Eimeria*** (*E. bovis* and *E. zuernii*) **in the sample, and semi-quantitative *Cryptosporidium* spp. OVA count** (oocysts per view area)

	All samples	*Eimeria* (OPG)		Pathogenic *Eimeria* species	*Cryptosporidium* spp. (OVA)		
		1-1000	>1000		1-5	6-25	>25
Diarrhoeic samples, % (95% CI)	10 (7-12)	4 (2-6)	2 (1-3)	4 (2-5)	2 (1-3)	4 (3-6)	4 (2-5)

Questionnaire

Seven farmers completed the questionnaire and were included in the analysis. No farmers had previously observed problems with parasites. Diarrhoea was observed by farmers (n=7): never 0%, rarely n=3 (43%; 95% CI 25-61), occasionally n=2 (29%; 95% CI 12-46), always n=2 (29%; 95% CI 12-46). The presence of diarrhoea was considered as a general problem by 3 farmers (43%; 95% CI 25-61). Only two farms had calves on pasture every year and both observed diarrhoea early after turnout. Calves were reared separate from older cattle in 5 (71%; 95% CI 54-88) of the farms.

Discussion

All the herds were positive for *Eimeria* spp. which correlates well with previous findings from Estonia, Lithuania, and Poland (Lassen et al., 2009a; Lassen and Järvis, 2009; Klockiewicz et al. 2007). The herd prevalence for *Cryptosporidium* spp. was slightly lower than in other Baltic countries where it reached almost 100%. Presumably, this and other differences discussed below were due to limited sample size, focus on larger herds, and narrow age-span of the animals sampled. The results from this study can, however, give the first look at the situation, by the documentation of specific *Eimeria* species and infection level in calves which may help to increase consideration on the parasites among farmers.

The prevalences of *Eimeria* and *Cryptosporidium* observed were between the prevalences reported from Estonia and Lithuania (Lassen and Järvis, 2009; Lassen et al., 2009a). In latter studies, older animals were also examined, in contrast to this study. This makes comparison difficult, since animals younger than 6 months are most susceptible to coccidiosis (Taylor and Catchpole, 1994). Latvian calves younger than 3 months of age were commonly infected with *Eimeria*: the prevalence (71%) was two and three times higher than those observed in Estonia and Lithuania, respectively. Within the same age group *Cryptosporidium* oocysts were found in 43% of the samples which is between the two neighbouring countries. These results indicate that a large proportion of animals in the risk group of developing disease due to infections with *Cryptosporidium* and *Eimeria* were infected with these species in Latvia. This may be important to consider since calves are most commonly shedding the zoonotic *C. parvum* genotype, and thus represent a potential health risk to humans in Europe (Chako et al., 2010).

Whether an oocyst count can be related to clinical signs of disease depends on additional factors such as parasite species present, age of the animal, and sampling in relation to the patency period (Daugschies and Najdrowski, 2005). Mundt and colleagues (2005) suggested that if *Eimeria* OPG counts are above 500, concomitant diarrhoea may be considered as coccidiosis related. In this study, the mean OPG counts were several times higher, but diarrhoea could not be related statistically to *Eimeria* infections or to OPG counts. Oocyst counts were especially high in the youngest age-group of calves. Experimental *Eimeria* infections have not shown a clear correlation between challenge dose and excretion of oocysts. Bangoura and Daugschies (2007) demonstrated with single experimental infections of 250000 *E. zuernii* oocysts that diarrhoea manifested with up to 100000 oocysts per gram faeces. Smaller and continuous challenge doses, however, may result in higher levels of oocysts in faeces compared with single high doses (Fitzgerald, 1967). The OPG count of over half a million *E. zuernii* observed in this study is considered exceptionally high. The calf survived the infection but the owner reported observing bloody diarrhoea in calves every time after regrouping them. In general, the OPG counts in Latvian herds were considerably higher when compared to those from Estonia and Lithuania (Lassen et al., 2009a; Lassen and Järvis, 2009).

Eight *Eimeria* species were identified in calves, and the most pathogenic were the dominating ones: *E. zuernii* and *E. bovis*. These were found occurring together in one third of the infections. The presence of the *E. zuernii* was similar to the other Baltic countries, but *E. bovis* was observed less frequently in Latvian farms (Lassen et al., 2009a; Lassen and Järvis, 2009). The highest oocyst counts were observed in samples where pathogenic species were predominant. Species not found in this study but observed in the other Baltic countries are likely to be present, and presumably could be isolated in a larger sample size. A difference was noted in number of species found in each sample. On average, two *Eimeria* species were found in positive samples from Latvia, while 6 and 7 species were identified in Estonian and Lithuanian samples, respectively. The combination of *E. zuernii* or *E. bovis* dominating in ¾ of the positive samples with high OPG counts means that a potential risk for clinical and subclinical disease is present. In addition, these findings may be of importance in evaluating the need to update the diagnostic procedures for coccidia in the Baltic countries. Considering the high herd prevalences, the currently used qualitative methods are of limited value. Although results from our study suggest that *Eimeria* positive samples are likely to contain a pathogenic species. Therefore it is paramount to consider each potential case of coccidiosis individually, with approach to identification of species, individual OPG counts, clinical signs, management, and the environment as a whole (Daugschies and Najdrowski, 2005).

Larger herds had less severe *Cryptosporidium* infections ($P<0.05$). This is in agreement with the results of an epidemiological study in large Estonian herds (Lassen et al., 2009b). Reasons for this may lie in management practices of larger farms.

Causes of diarrhoea in calves are often complex and may involve age, feed changes, stress, and several pathogens such as *Escherichia coli* F5, rotavirus, bovine coronavirus, *Cryptosporidium*, and *Eimeria* (Krogh and Henriksen, 1985; De Rycke et al. 1986; De la Fuente et al. 1999; Daugschies and Najdrowski, 2005). Nevertheless, diarrhoea is one of the best indicators of clinical disease caused by both *Eimeria* and *Cryptosporidium* (Tzipori et al. 1983; Cornelissen et al. 1995. In this study, common causes of diarrhoea in calves other than *Cryptosporidium* and *Eimeria* were not considered, and the statistical associations should be interpreted with this in mind. Diarrhoea was not observed frequently but was indicating a statistical correlation between the occurrence of diarrhoea in calves and *Cryptosporidium*. *Eimeria* infection could not be associated with watery faeces in this study, and faeces with high oocysts counts were generally not diarrhoeic. Similar findings have been reported previously by Gulliksen et al. (2009) and Lassen et al. (2009a).

Latvian farmers did not consider parasites as causing clinical disease in their farms, but did observe diarrhoea in indoor and pastured calves. This is in correspondence with reports from Estonian dairy farmers (Lassen et al., 2009a). In this study, we could not examine the prevalence of clinical coccidiosis in Latvian dairy farms. However, the observations of bloody diarrhoea in farms with heavy *E. zuernii* infections indicate that eimeriosis may be of clinical importance in Latvian dairy farms.

The small variation of species, high prevalence of pathogenic species, and high OPG counts suggest that pathogenic *Eimeria* infections are potentially of higher importance in Latvia when compared with other Baltic countries. *Cryptosporidium* is also commonly present in Latvian cattle herds. Pathogenic coccidian species are likely to be responsible for clinical infections however are of low awareness among Latvian farmers.

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