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A SEVEN-YEAR STUDY ON THE PREVALENCE AND INTENSITY OF *CRYPTOSPORIDIUM* SPP. INFECTIONS IN DAIRY CATTLE IN LATVIA: REGIONAL AND AGE-RELATED VARIATIONS

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Abstract
This study investigates the prevalence and intensity of *Cryptosporidium* spp. infections in dairy cattle across Latvia, focusing on regional and age-related variations. Over the period from 2013 to 2020, fecal samples from 2,655 dairy cattle were analyzed using Ziehl-Neelsen staining technique and flotation methods. The overall prevalence of *Cryptosporidium* spp. was found to be 27%, with significant regional differences, the highest prevalence observed in the Vidzeme region (31%) and the highest oocyst counts in the Kurzeme region (median = 600 OPG). Age-related susceptibility was evident, with calves aged 0 to 3 months showing the highest infection rates (39.4%) and oocyst counts (median = 800 OPG). Diarrhea was significantly more common in infected calves (56.6%) compared to older cattle. The findings highlight the need for targeted interventions in young calves and region-specific control strategies to mitigate the impact of cryptosporidiosis on the dairy industry. This comprehensive study provides valuable insights into the epidemiology of *Cryptosporidium* spp. in Latvian dairy cattle, emphasizing the importance of age and regional factors in infection dynamics.

Keywords: *Cryptosporidium* spp., Latvia, epidemiology, regional variation, age-related.

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INTRODUCTION

The study of *Cryptosporidium* spp. in dairy cattle has garnered significant attention due to its implications for both human health and animal productivity. *Cryptosporidium* spp. are protozoan parasites that infect the gastrointestinal tract of various hosts, leading to cryptosporidiosis, a disease characterized by diarrhea and other gastrointestinal symptoms (Fayer et al. 2000). The prevalence and intensity of *Cryptosporidium* infections in livestock, particularly dairy cattle, have been extensively documented, highlighting the economic and health burdens associated with these infections (Santín 2013). Therefore, understanding the epidemiology of *Cryptosporidium* spp. in dairy cattle is crucial for developing effective control and prevention strategies, which can mitigate the impact of this parasite on the dairy industry.

Previous research has demonstrated that the prevalence of *Cryptosporidium* spp. in dairy cattle varies widely across different regions and management practices (Xiao & Fayer 2008, Thomson et al. 2017). Factors such as age, environmental conditions, and herd management practices significantly influence the infection rates and oocyst shedding in cattle (Thomson et al. 2017). Young calves are particularly susceptible to infection, often exhibiting higher prevalence rates and more severe clinical symptoms compared to older cattle. Zambriski et al. (2013) showed that 17 oocysts were sufficient to cause diarrhea and oocyst shedding. Research shows that the infection rate in calves can reach up to 79.5%, significantly higher than in adult cattle (Olson et al. 2004, Bartley et al. 2023). This age-related susceptibility underscores the need for targeted interventions in young animals to reduce the overall burden of cryptosporidiosis in dairy herds.

The detection and quantification of *Cryptosporidium* oocysts in fecal samples are critical for assessing infection dynamics and implementing control measures. Various diagnostic techniques, including microscopy, immunoassays, and molecular methods, have been employed to identify and quantify *Cryptosporidium* oocysts in cattle feces (Smith et al. 2007, Vanathy et al. 2017). Among these, the modified Ziehl-Neelsen staining technique and flotation methods are commonly used due to their reliability and cost-effectiveness (Kuczynska & Shelton 1999). Accurate detection and quantification are essential for epidemiological studies, which provide insights into the distribution and intensity of infections within and between herds.

The present study aims to investigate the prevalence and intensity of *Cryptosporidium* spp. infections in dairy cattle in Latvia, with a focus on regional variations and age-related differences. Previous studies in Latvia have been limited in sample size, making this research the most comprehensive investigation of *Cryptosporidium* spp. prevalence and intensity of infection. By employing established diagnostic methods and statistical analyses, this research seeks to contribute to the existing body of knowledge on *Cryptosporidium* epidemiology in dairy cattle.

MATERIAL AND METHODS

The investigation was conducted over the period from 2013 to 2020. A total of 2655 dairy cattle were subjected to testing for the presence of *Cryptosporidium* spp. parasites through the analysis of fecal specimens collected during routine health assessments. In most cases, individual fecal specimens were obtained directly from the rectum. In exceptional circumstances, specimens were procured during the defecation process or retrieved from the ground immediately following the excretion by the animal. Specimens were gathered utilizing disposable gloves and subsequently maintained in a portable cooler during transit to the laboratory, where they were stored at a temperature of +4 ^{0}C until they underwent examination.

Samples intended for conventional microscopy

were subjected to a saturated NaCl flotation methodology (Kuczynska & Shelton 1999). In the flotation procedure, a quantity of 1 g of fecal sample was utilized, and following the flotation and centrifugation procedures, 2 ml of purified material was acquired for subsequent analytical assessments. Initially, all samples of the purified material underwent an examination. A 10 µl droplet of the purified oocysts was placed upon a microscopic slide, allowed to air dry at ambient temperature, and subsequently stained utilizing a modified Ziehl-Neelsen technique. All oocysts exhibiting a dark red to pink coloration with characteristic morphology were counted for each of the 10 µl droplets employing a microscopy magnification of 200x. Consequently, each oocyst identified microscopically corresponded to an equivalent of 200 oocysts per gram of feces (OPG) (Åberg et al. 2019).
The prevalence was calculated as the proportion of dairy cattle individual in a population infected with a *Cryptosporidium* spp. parasites. The comparison of prevalence and diarrhea between age groups was conducted by using the chi-squared test of homogeneity. The comparison of OPG between age groups was processed by the Kruskal – Wallis H test. Statistical analyses were conducted using Jamovi (v 2.5.5). Results were considered statistically significant at a p-value of less than 0.05.

RESULTS

The total prevalence of *Cryptosporidium* spp. infection in Latvia was determined to be 27% and a 95% confidence interval ranging from 26% to 29% with a median oocyst count per gram measured at 1000 (ranging from Q1 = 400 to Q3 = 3000). Diarrhea was observed in 30.1% of cows infected with *Cryptosporidium* spp., and it was noted in 24.5% of cows that were not infected with *Cryptosporidium* spp.
The minimum oocyst count per gram identified in bovine feces was 200, while the maximum count reached 476,500. The recorded oocyst counts per gram adhere to a negative binomial distribution, exhibiting a substantial degree of clustering or overdispersion, which suggests that the distribution of oocysts is not entirely stochastic. The majority of the analyzed fecal samples contained between 200 and 2000 oocysts per gram, with only a limited number of samples exceeding 15,000 oocysts, thereby underscoring a significant variation in infection intensity (Fig. 1).

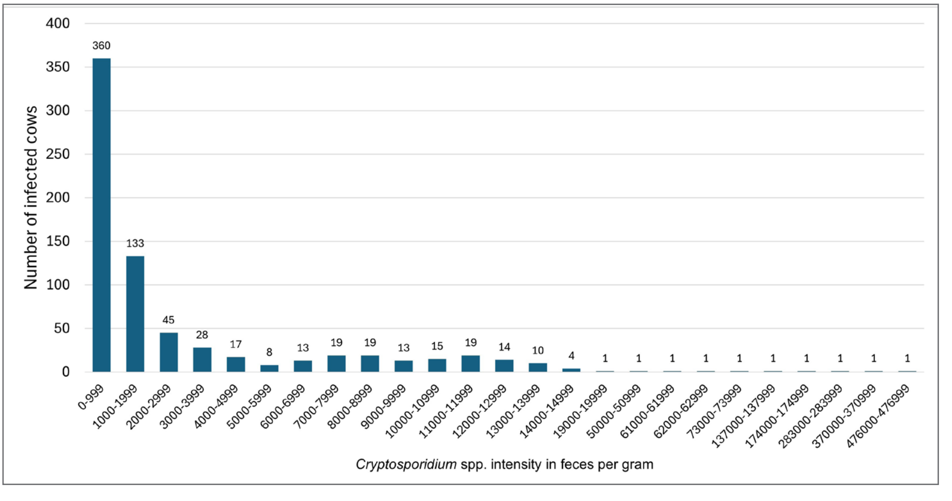

Figure 1. Distribution of *Cryptosporidium* spp. oocysts of bovine feces in Latvia.

The prevalence and oocyst count per gram of bovine feces exhibited a non-random distribution across planning regions of Latvia, showing the highest prevalence in Vidzeme region (31%) and highest oocyst count in Kurzeme region (median = 600, Q1-Q3 300 – 1200). The distribution of *Cryptosporidium* spp. with intensity of infection in Latvia by planning regions has been summarized in the Fig. 2.

The comparison of *Cryptosporidium* spp. infection revealed a statistically significant difference of prevalence between age groups ($p < 0.001$) and demonstrated a considerable reduction in infection rates as bovines mature (Tab. 1). Furthermore, the examination of diarrhea in cows infected with *Cryptosporidium* spp. parasites revealed a statistically significant difference among age groups ($p < 0.001$), with the highest percentage observed in calves aged 0 to 3 months.

Table 1. Mean prevalence, diarrhea and intensity of *Cryptosporidium* spp. infection in cows by age groups.

Parameter	Prevalence, % (95% CI)	Median (Q1 – Q3)	Diarrhea with *Cryptosporidium* spp., % (95% CI)
From 0 to 3 months	39.4 (32.6 – 46.5)	800 (200 – 2400)	56.6 (44.7 – 67.9)
From 4 to 24 months	20.3 (17.0 – 23.9)	400 (400 – 650)	4.2 (0.1 – 21.1)
More than 24 months	19.2 (16.7 – 21.9)	600 (400 – 1000)	7.0 (1.9 – 17.0)
p-value	<0.001	0.118	<0.001

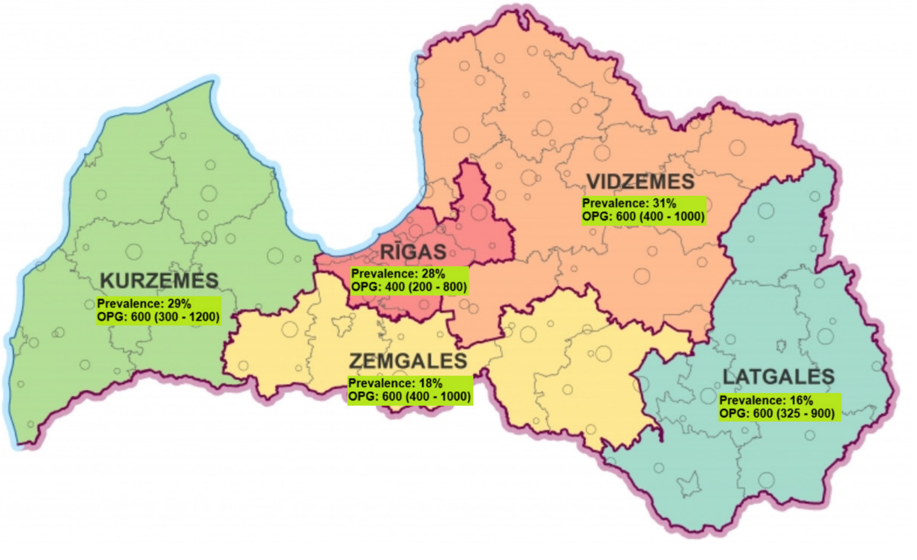

Figure 2. Mean prevalence (%) and median (Q1 – Q3) of *Cryptosporidium* spp. oocyst per gram of bovine feces (OPG) by planning regions of Latvia (according to the Cabinet of Ministers' regulations of June 22, 2021, No. 418). Source: https://www.varam.gov.lv/lv/planosanas-regioni.

DISCUSSION

This study represents the first extensive, long-term investigation of *Cryptosporidium* spp. infections in Latvian dairy cattle, encompassing a large sample size of over 2,500 animals. The findings of this study reveal a significant prevalence of *Cryptosporidium* spp. infections in dairy cattle across Latvia, with notable regional and age-related variations. The reported overall *Cryptosporidium* prevalence rate of 27% in dairy herds is consistent with findings from various studies, which indicate similar infection rates across different regions. For instance, a meta-analysis revealed a global prevalence of 25.5% in cattle, with significant variations depending on geographical location and management practices (Buchanan et al. 2024). Specific studies have shown rates of 28.7% in India (Agrawal et al. 2023), 30% in France (Certad et al. 2024), and even higher rates in certain populations of calves, such as 91.6% in a Slovakian dairy farm (Kaduková et al. 2024). These findings underscore the nature of *Cryptosporidium* infections in dairy cattle and highlight the importance of monitoring and management practices to mitigate the economic and public health impacts associated with this zoonotic pathogen. Moreover, the highest prevalence observed in the Vidzeme region and the highest oocyst counts in the Kurzeme region suggest that environmental and management factors may play a crucial role in the distribution and intensity of infections.

The age-related differences in *Cryptosporidium* infection rates and oocyst shedding observed in this study are particularly noteworthy. Calves aged 0 to 3 months exhibited the highest prevalence and oocyst counts, which is in line with previous research indicating that young calves are more susceptible to *Cryptosporidium* infections. For example, Aguilar reported a prevalence of 26.6% in calves from dairy herds in Colombia, with the highest infection rates observed in calves aged 8-14 days, indicating a critical vulnerability during this early life stage (Aguilar 2023). Similarly, Urie et al. found that younger calves were more likely to test positive for *Cryptosporidium*, with peak prevalence occurring around two weeks of age (Urie et al. 2018). This aligns with findings from Garro et al., who noted that the frequency of oocyst shedding was highest in calves under 20 days of age (Garro et al. 2016). Doungmala et al. highlighted that oocyst shedding was particularly pronounced in calves aged 1-3 weeks, reinforcing the notion that early exposure is critical for infection (Doungmala et al. 2019). Additionally, Ebiyo and Haile demonstrated that calves under six months had a significantly higher risk of infection, with an odds ratio of 2.7, emphasizing the importance of age as a risk factor (Ebiyo & Haile 2022). The significant reduction in infection rates and oocyst shedding in older cattle underscores the importance of implementing targeted interventions in young calves to mitigate the impact of cryptosporidiosis in dairy herds.

The association between *Cryptosporidium* infections and diarrhea in dairy cattle observed in this study further underscores the clinical significance of this parasite. The higher incidence of diarrhea in infected animals, particularly in young calves, aligns with the pathogenic potential of *Cryptosporidium* spp. to cause gastrointestinal disturbances (Fayer et al. 2000). For example, Wells et al. reported a high prevalence of *Cryptosporidium* in cattle during the calving season, suggesting that the timing of calving significantly influences infection rates and, consequently, the incidence of diarrhea (Wells et al. 2015). Thomson et al. reported that *Cryptosporidium* was the most commonly detected pathogen causing diarrhea in calves less than one month of age, further highlighting its impact on animal health (Thomson et al. 2017). This age-related susceptibility is critical for understanding the dynamics of *Cryptosporidium* transmission within herds and for developing effective management strategies. Moreover, the economic implications of *Cryptosporidium* infections are substantial. Diarrhea in calves can lead to dehydration, weight loss, and increased veterinary costs, ultimately affecting the productivity of dairy operations. As highlighted by Berhanu et al., adult cattle can serve as reservoirs for *Cryptosporidium*, shedding oocysts that contaminate the environment and pose a risk to younger animals (Berhanu et al. 2022). This emphasizes the need for comprehensive herd management practices that address both adult and juvenile cattle to mitigate the risk of outbreaks.

The detection of *Cryptosporidium* oocysts using the modified Ziehl-Neelsen staining technique and flotation methods proved effective in this study, showing the reliability of these diagnostic approaches (Kuczynska & Shelton 1999). The negative binomial distribution of oocyst counts highlights the overdispersion and clustering of infections within the population, which has been observed in other parasitological studies. For example, the negative binomial distribution is widely recognized for its ability to model the aggregated distribution of parasites among hosts, where a few hosts harbor many parasites while most hosts have few or none (Ieshko et al. 2024). This distribution is often attributed to variations in host susceptibility and parasite exposure (Gourbière et al. 2015). This suggests that a small proportion of highly infected animals may contribute disproportionately to environmental contamination and transmission dynamics, emphasizing the need for targeted control measures.

CONCLUSIONS

In conclusion, this study provides valuable insights into the epidemiology of *Cryptosporidium* spp. infections in dairy cattle in Latvia. The regional and age-related variations in prevalence and oocyst shedding underscore the need for tailored control strategies that consider local environmental conditions and the specific vulnerabilities of different age groups. Future research should focus on elucidating the specific environmental and management factors that contribute to the observed variations in infection rates, as well as developing and implementing effective intervention strategies to reduce the burden of cryptosporidiosis in dairy herds.

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