Iraqi Journal of Veterinary Sciences

www.vetmedmosul.com

Impact of farm management practices on the prevalence of *Cryptosporidium* infection in neonatal calves in Denizli province, Turkey

Z.T. Cinar[1], O.S. Aldemir[2] and S. Bakirci[2]

[1]Denizli Directorate of Provincial Agriculture and Forestry, Ministry of Agriculture and Forestry, Denizli, [2]Department of Parasitology, Faculty of Veterinary Medicine, Aydın Adnan Menderes University, Aydın, Turkey

Article information

Article history:
Received 17 May 2025
Accepted 18 September 2025
Published 01 November 2025

Keywords:
Cryptosporidium
Epidemiology
Neonatal calf
Risk factors
Zoonotic disease

Correspondence:
Z.T. Cinar
zynptoprak@gmail.com

Abstract

This study aimed to evaluate the prevalence of *Cryptosporidium* spp. in neonatal calves and its association with farm management practices. The research was conducted on 26 cattle farms located in Denizli Province, Türkiye, by collecting fecal samples from a total of 250 neonatal calves. The samples were examined using Kinyoun's acid-fast staining, followed by nested PCR methods, and the prevalence of *Cryptosporidium* spp. was determined to be 38.8% and 68.4%, respectively. Farm management data were collected and analyzed using the Pearson chi-square test. The infection rate was found to be higher in calves aged 1-10 days 84% and in female calves 87.4%. Additionally, a lower infection rate 46.2% was observed in calves administered with hyperimmune serum at birth. As a result, nested PCR methods demonstrated higher sensitivity compared to Kinyoun's acid-fast staining. The prevalence of *Cryptosporidium* spp. in neonatal calves in Denizli was found to be remarkably high 68.4%, highlighting the necessity for improved farm management. The diversity of infection-associated factors complicates the planning of preventive veterinary practices. Due to the zoonotic nature of *Cryptosporidium*, a "One Health" strategy should be adopted, and more comprehensive and sustainably coordinated public health and biosecurity measures should be taken to minimize infection rates and protect both animal and human health.

DOI: 10.33899/ijvs.2025.165197.4515,

Introduction

The neonatal period (0 to 28 days) represents the most critical phase in calf rearing (1) and directly influences future productivity parameters of the animals (2). Scours is commonly observed during this stage (3). Although its long-term consequences are not fully clarified, neonatal scours may significantly reduce the average daily weight gain of calves, particularly during the first six months of life (4). Calf scours represent a significant health challenge for dairy and beef farms globally, as they are one of the most prevalent diseases in pre-weaned calves, leading to high treatment rates and considerable mortality. Among the various pathogens implicated in neonatal calf diarrhea, *Cryptosporidium* spp., rotavirus, coronavirus, and *Escherichia coli* are the most commonly identified causative agents (5). *Cryptosporidium* is a zoonotic protozoan parasite that can spread from human to human or animal to animal (anthroponotic transmission) or from animal to human (zoonotic transmission) and is important for both human and animal health worldwide (6). It infects the digestive system of all types of vertebrates, causing stomach problems and significant financial losses (7,8). *Cryptosporidium* has been designated as a "neglected pathogen" by the World Health Organization (WHO) within its 2004 Neglected Diseases Initiative. The significance of this protozoan has become increasingly recognized (9). *Cryptosporidium* species have attracted more attention due to their periodic outbreaks, zoonotic potential, clinical relevance in immunodeficient individuals, and the considerable economic losses they

cause, particularly in newborn calves (10). Mortality resulting from calf scours causes significant financial losses. For example, annual losses in Norway have been estimated at approximately 10 million USD (11). In Türkiye, it is estimated that at least 15% of neonatal calves are lost during this period, with economic losses reaching approximately 3.15 billion TRY in 2018 alone (12). The persistence of *Cryptosporidium* in the environment and its resistance to conventional water treatment methods further complicate efforts to control its spread, highlighting the need for improved public health strategies and research into effective treatment options (13). Understanding these dynamics is crucial for developing targeted interventions that can reduce transmission risks. Collaborative efforts between the veterinary and public health sectors will be essential in formulating comprehensive strategies to combat the challenges posed by *Cryptosporidium* (14).

Although the exact neonatal calf mortality rate in Türkiye remains unclear, it is assumed to be high. Nevertheless, data on the prevalence of *Cryptosporidium* infections in neonatal calves and their association with farm management practices are scarce. This lack of information hinders the implementation of effective preventive measures and limits the control of zoonotic transmission risks. Therefore, the present study aims to determine the prevalence of *Cryptosporidium* spp. in neonatal calves and to investigate its relationship with farm management practices. In this context, the findings are expected to contribute to the development of farm management strategies and biosecurity measures and to support national and international efforts for controlling zoonotic infections. Furthermore, although the study was conducted in a limited geographical area, it represents the first large-scale investigation in Türkiye focusing on the relationship between *Cryptosporidium* prevalence and farm management practices.

Materials and methods

Ethical approval

This study was approved by the Local Ethics Committee for Animal Experiments of Aydın Adnan Menderes University (Decision No: 64583101/2021/040, 2021 - Session III). The present study was conducted in 2021 as part of a doctoral thesis project, and the data collection period reflects the timeline of the original research design approved by the institutional ethics committee.

Study area and sample collection

This descriptive study was conducted on cattle farms located in the Denizli province of Türkiye (Figure 1). A total of 250 fecal samples were collected from neonatal calves aged 0-28 days. The sample size was initially calculated as 345 based on the epidemiological formula defined by Martin *et al*. (15) ($n=4\times P\times Q/L^2$); however, it was limited to 250 due to logistical and economic constraints. The study was carried out on 26 farms selected based on herd size, hygiene practices, and calf housing conditions. Fecal samples were collected via rectal stimulation (16). Additional data were recorded regarding calf age, sex, breed, health status, timing and duration of colostrum intake, feeding programs, housing conditions, drug or vaccine administration, total herd and calf numbers, vaccination practices, and farm-level sanitation and disinfection procedures.

Figure 1: Distribution of calf samples by location from farms in Denizli province, Türkiye. Location of the study area.

Laboratory analyses

For microscopic examination, smears prepared from fecal samples were fixed with methanol, stained using Kinyoun's acid-fast staining method, and examined at 100× magnification with an Olympus BX51 microscope (17). Images were captured using a DP70 camera. DNA extraction was performed using the Exgene™ Stool DNA kit according to the manufacturer's protocol, and samples were stored at −20 °C. PCR amplification targeted a 1325 bp fragment of the SSU-rRNA gene using primers Crypto F1 and R1 (18). Nested PCR was subsequently conducted using primers specific to the 826-864 bp region (19). Amplifications were carried out in 30 µl volumes using a SimpliAmp Thermal Cycler. Final products were separated by agarose gel electrophoresis and visualized under UV light with the UVP EC3 Chemi HR410 Imaging System.

Statistical analysis

Statistical analysis was performed using the IBM SPSS Statistics software (version 25). Descriptive statistics for categorical variables were presented as counts and percentages. The relationships between diagnostic results and farm management variables were analyzed using the Pearson chi-square test. A 95% confidence level was used, and p-values less than 5% were considered statistically significant.

Results

Cryptosporidium was detected in 171 out of 250 samples examined, resulting in an overall prevalence of 68.4% based on nested PCR results (Figure 2). A statistically significant

association was observed between microscopic staining and molecular analysis (P=0.000; P<0.05), as all microscopically positive samples were confirmed by molecular methods, while 74 microscopically negative samples were identified as positive by nested PCR (Table 1). Among the host-related variables, only calf age, sex, and *E. coli* hyperimmune serum administration were found to be significantly associated with infection (P<0.05) (Table 2). In contrast, no significant associations were observed between *Cryptosporidium* positivity and the non-host-related factors such as herd size, season, feeding practices, housing conditions, or sanitation measures (Table 3).

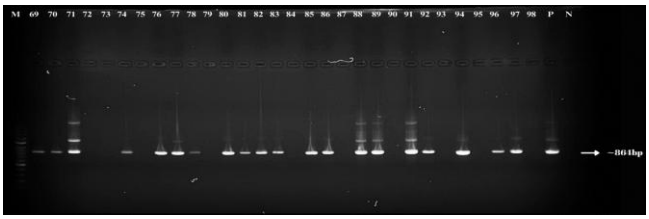

Figure 2: Agarose gel electrophoresis image of nested PCR products (original). M: DNA marker (100 bp); P: known positive control; N: known negative control.

Table 1: Distribution of the rates of kinyoun's acid-fast staining results and nested PCR data

Microscopic examination	Nested PCR result		Total	%
	Positive	Negative		
Positive	97	0	97	100
Negative	74	79	153	48.4
Total	171	79	250	68.4

Discussion

Compared to microscopic techniques, molecular methods are known to have higher sensitivity for detecting *Cryptosporidium* oocysts (19). In our study, every sample identified as positive by microscopic examination was also confirmed positive by molecular analysis. Additionally, *Cryptosporidium* spp. was detected exclusively by molecular methods in 74 samples. This finding confirms the ability of PCR to detect low oocyst concentrations.

The lack of a statistically significant relationship between diarrhea and *Cryptosporidium* reflects the conflicting findings in the literature. While some studies have reported a strong association between diarrhea and *Cryptosporidium* infection (20), others have identified high prevalence even in clinically healthy calves (21). In our study, the prevalence of *Cryptosporidium* was found to be equal in both diarrheic and non-diarrheic neonatal calves (Table 2). These results suggest that, although *Cryptosporidium* spp. may be an important pathogen responsible for diarrhea, stool consistency cannot reliably be used as an indicator of its presence.

Herd size is considered another potential risk factor for *Cryptosporidium* infection (22). Some studies have suggested that calves in small-scale farms may shed more oocysts compared to those in larger operations (23) or that the increased contact among calves in large herds elevates the risk of infection (24). However, other studies have found no association between larger herd size and increased infection risk (25). Similarly, in our study, no direct relationship was identified between herd size and infection rate; the highest infection rate was observed in medium-sized herds (51-100 head) at 76.9% (Table 3). Therefore, the influence of herd size on infection risk cannot be evaluated in isolation and should be considered alongside other factors such as farm management practices, animal density, nutrition, water sources, and age.

Seasonal variation is considered an influential factor in the spread of *Cryptosporidium* infections (22). Several studies have reported a higher incidence of calf diarrhea during the winter and spring months (26). However, it has also been suggested that this increase may be due to the overlap between calving periods and specific seasons (27). In farms with year-round calving, the infection is more prevalent during the summer months, which is thought to be associated with elevated temperatures and humidity (28). Some studies have linked the frequency of infection not directly to seasonality, but rather to the amount of precipitation (29). In the present study, the highest positivity rate was observed in the autumn months (76.6%) (Table 3); however, during the sampling period, average temperatures were high, and precipitation was low. Consequently, this study, in line with similar findings in the literature, suggests that *Cryptosporidium* infection can be prevalent throughout the year and that seasonal variability may not play a decisive role (30).

As reported in various studies, no significant association was found between calf breed and *Cryptosporidium* infection rate in this study (31). However, some studies have indicated that pure breeds may be at higher risk compared to crossbreeds (32) and that Holstein calves may have a greater likelihood of being infected with *C. parvum* (33). In this study, although the infection rate among Montbeliarde calves appeared to be 100%, only two calves of this breed were included. The other breeds showed comparable levels of infection risk (Table 2).

In our study, the infection rate in female calves (87.4%) was notably higher compared to male calves (Table 2). The literature contains conflicting findings regarding the relationship between sex and *Cryptosporidium* infection; some studies have reported higher prevalence in male calves (34), while others have found no significant association (35). These discrepancies may be attributed to various factors, including sample size, characteristics of the calf population, methodologies employed, potential differences in immune responses of female calves, or differences in management practices (e.g., housing, feeding).

The most significant risk of mortality in calves occurs during the first 21 days of life (36). According to the United States National Animal Health Monitoring System, 57% of calf deaths are attributed to diarrhea (37). In cases where *C. parvum* is identified as the sole pathogen, high mortality rates have been observed in association with prolonged and persistent diarrhea (38). In our study, six calves died during the neonatal period, and four of them were found to be infected with *Cryptosporidium* spp., exhibiting severe diarrhea.

Table 2: Distribution of *Cryptosporidium* spp. positivity in neonatal calves according to host-related variables (n=250; based on nested PCR result

Variable	Category	PCR (n)		+ (%)	P
		+	-		
Feces consistency	Diarrhea	26	12	68.4	0.998
	Normal Consistency	145	67	68.4	
Calf age	1-10 days	79	15	84	0.000
	11-20 days	66	21	75.9	
	21-29 days	26	43	37.7	
Breed	Simmental	39	20	66.1	0.537
	Holstein	124	54	69.7	
	Danish Red	6	5	54.5	
	Montbeliard	2	0	100	
Sex	Female	118	17	87.4	0.000
	Male	53	62	46.1	
Colostrum intake	2 days	73	37	66.4	0.539
	3 days	98	42	70	
Colostrum first intake	Uncertain	18	4	81.8	0.415
	First 12 hours + every 8 hours thereafter	86	41	67.7	
	First 12 hours + every 12 hours thereafter	61	29	67.8	
	Immediately after birth	6	5	54.5	
Colostrum amount	Uncertain	18	4	81.8	0.449
	First (2-2,5 lt) + 1-1,5 lt	5	1	83.3	
	2,5 lt + 2,5 lt	2	0	100	
	First (4-4,5 kg) + 2-2,5 kg	8	5	61.5	
	First (4-4,5 kg) + 1-1,5 kg	132	64	67.3	
	First (5-6 lt) + Uncertain	6	5	54.5	
Colostrum delivery	Directly from the dam	18	4	81.8	0.221
	Bottle	151	75	66.8	
	Bucket	2	0	100	
Hyperimmune serum	Application as soon as born	12	14	46.2	0.010
	No app	159	65	71	
Treatment/Supplement	Özisdur (First 3 days)	2	0	100	0.179
	No treatment applied	113	57	66.5	
	Amoxicillin	1	0	100	
	Enrofloxacin	0	2	0	
	Novostrum (D1), Zactran (D2), Halofuginone lactate (D3-8)	6	5	54.5	
	Trimethoprim-sulfonamide	1	0	100	
	Tetracycline + fluid therapy	1	0	100	
	Fluid therapy + multivitamin	0	1	0	
	Fluid therapy + multivitamin + tetracycline	0	1	0	
	Fluid therapy + Ceftiofur	2	0	100	
	Parofor liquid	4	1	80	
	Vitamin D, Iron, Macrolide + Sulfonamide	6	4	60	
	Injocam C	26	5	83.9	
	Yeldif	9	3	75	
Mortality	Deceased during the neonatal period	4	2	66.7	0.926
	Survived during the neonatal period	167	77	68.4	

Table 3: Distribution of *Cryptosporidium* spp. positivity in neonatal calves according to non-host-related variables (n=250; based on nested PCR result).

Variable	Category	PCR (n)		+ (%)	P
		+	-		
Herd size	Between 1-20 heads	9	3	75	
	Between 21-50 heads	16	12	57.1	
	Between 51-100 heads	20	6	76.9	0.287
	Between 101-200 heads	12	10	54.5	
	Between 201-500 heads	114	48	70.4	
Month	March-April-May	49	34	59	
	June-July-August	86	34	71.7	0.067
	September-October	36	11	76.6	
Feed	Calf Starter Feed + Oats	2	0	100	
	Calf Starter Feed + Sprout	3	0	100	
	Calf Starter Feed	38	31	55.1	
	Milk curdled with formic acid + Calf Starter Feed	6	5	54.5	0.018
	Calf Starter Feed + Same feed as mother after 7th day	51	16	76.1	
	Calf Starter Feed + roughage	45	11	80.4	
	Concentrate Feed	26	16	61.9	
Hygiene	No hygiene/sanitation measures applied	111	49	69.4	
	Water cleaning every three days; Virkon S and Prophyl S every two weeks	25	11	69.4	0.765
	Cleaning of bedding in pens every three days	26	16	61.9	
	Removal of feces and cleaning with pressurized water	9	3	75	
Housing	Indoor housing with a separate calf section; poor bedding	2	0	100	
	Semi-open housing with a separate calf section; poor bedding	6	1	85.7	
	Indoor housing without a separate calf section; poor bedding	2	0	100	
	Indoor housing without a separate calf section; clean bedding	4	1	80	
	Indoor housing, the calf area separated by a partition; poor bedding	1	2	33.3	
	Indoor housing, calves kept in separate, elevated compartments; clean bedding	6	5	54.5	
	Indoor housing without partitions; clean bedding conditions	1	1	50	
	Indoor housing with adjacent individual compartments; poor bedding	5	3	62.5	
	Semi-open housing, calves kept in separate sections; poor bedding	2	1	66.7	0.271
	Semi-open housing without partitions; wet bedding	2	1	66.7	
	Indoor housing with side-by-side compartments; clean bedding	27	10	73	
	Semi-open housing with adjacent individual pens; clean bedding	17	14	54.8	
	Calf hutches arranged adjacently; clean bedding; hotel system	25	11	69.4	
	Semi-open housing with separate, adjacent wooden pens; poor bedding	9	0	100	
	Indoor housing with a single compartment dedicated to calves; clean bedding	0	2	0	
	Semi-open, side-by-side pens separated by concrete walls; poor bedding	26	16	61.9	
	Semi-open, calves separated by iron partitions according to age groups	27	8	77.1	
	Open housing; bedding composed of straw and corn is wet and in poor condition	9	3	75	

There are conflicting findings in the literature regarding the protective efficacy of *E. coli* vaccination against infection (24). While one study by Trotz-Williams *et al*. (39) reported that *E. coli* vaccination reduced *C. parvum* oocyst shedding, another study found that it increased the risk of disease (39), and yet another reported no effect at all (40). In our study, a lower infection rate (46.2%) was observed in calves that received *E. coli* hyperimmune serum immediately after birth (Table 2). This protective effect may be associated with the prevention of potential secondary infections caused by *E. coli*, the absence of additional infections that cause damage to the intestinal epithelium, or general support of the immune system.

Prophylactic vitamin supplementation is recommended for calves (41). However, some studies suggest that the administration of vitamin E, selenium, antibiotics, additives, and antimicrobials through liquid diets does not significantly affect disease risk (42). In our study, no oocysts were detected in calves that received fluid therapy, multivitamins, tetracycline, and Setifour (Table 2), which is a noteworthy finding. Nevertheless, due to the limited number of such cases, further research is needed to generalize this relationship.

Paromomycin, used against *C. parvum* infection, is recommended both therapeutically and prophylactically due to its effect in reducing oocyst shedding and shortening the

duration of diarrhea (43). "Parofor Crypto" should be administered at a dose of 2.5 ml per 10 kg body weight for 7 consecutive days, only to animals with a confirmed *Cryptosporidium* diagnosis and before the onset of diarrhea (44). In our study, infection was detected in 4 out of 5 calves that received "Parofor Crypto" treatment (Table 2); however, it was reported that the treatment was discontinued after only three days upon improvement of clinical signs. Therefore, no definitive conclusion can be drawn regarding its effectiveness.

Halofuginone lactate acts on the merozoite and sporozoite stages of *C. parvum*, reducing the severity of cryptosporidiosis, delaying the onset of infection, and decreasing oocyst shedding. It is therefore recommended as both a therapeutic and prophylactic agent for calves (45). It has also been reported to reduce mortality (46,47). In our study, the infection rate was found to be 54.5% in a farm where halofuginone lactate was routinely administered to neonatal calves between days 3 and 8 postpartum (Table 2). However, the success of this application depends not only on pharmacological intervention but also on the integrated management of individual housing, hygiene, and environmental factors (48).

Neutralizing antibodies present in colostrum have been reported to reduce the infectivity of *Cryptosporidium* by preventing the adhesion of sporozoites to host cells and may even exhibit direct cytotoxic effects (49). Calves fed with colostrum from dams possessing high titers of specific antibodies are partially protected against infection (50). Therefore, feeding with high-quality colostrum and ensuring passive transfer is considered a critical management practice to minimize protozoal infections (51). Moreover, *Cryptosporidium* infections have been reported to be more severe in calves with failure of passive transfer (52).

On the other hand, some studies have shown that colostral immunoglobulins have limited effectiveness in preventing infection (39). Although there is weak evidence suggesting that colostrum may have a protective role, colostrum intake has not yet been effectively tested as either a risk or protective factor (24). In the present study, high rates of oocyst detection were also observed in calves that had received colostrum, indicating no significant association between colostrum intake and infection (Table 2). This finding may be explained by the intracellular nature of *C. parvum*, which allows it to evade antibody-mediated effects within the intestinal epithelial cells (43).

Pooling colostrum from multiple cows is generally thought to reduce overall colostrum quality and increase the risk of disease transmission (51). However, there is no consistent evidence regarding the impact of colostrum feeding methods on infection risk (24). Trotz-Williams *et al*. (39) and Silverlas *et al*. (53) reported that bottle feeding had no significant effect on the risk of infection. Nevertheless, milk contamination is possible through contaminated bottles or during milking (54). In our study, the infection rate among calves fed with a bottle was 66.8%, whereas it was 81.8% among those allowed to nurse directly from the dam (Table 2). This may be explained by the direct exposure to a high number of oocysts through contact with teats contaminated by feces. The use of nipple-less buckets to feed calves is thought to increase the risk of oocyst shedding, as it fails to satisfy the calves' natural suckling behavior (55). Although all calves fed with buckets in our study were found to be oocyst-positive, only two calves were fed this way; thus, the results are not generalizable (Table 2).

The source and handling of feed can influence *Cryptosporidium* infection risk (6). Feeding starter grains in addition to milk has been linked to higher oocyst shedding than milk replacers alone (56). In contrast, contamination of improperly prepared replacers may also contribute to infection (57). Poor-quality replacers can impair immune function and increase susceptibility (58). However, evidence regarding milk replacer use remains inconsistent (40,42), and further investigation is warranted (24). In our study, no significant association was observed between the use of supplemental feeds and the prevalence of *Cryptosporidium* infection. However, the lowest infection rate was detected in calves that were fed with cold milk treated with formic acid and calf starter feed. It is important to note that this feeding practice was implemented in only one farm (Table 3).

Disinfectants containing hydrogen peroxide and quicklime can reduce oocyst counts (52); however, in this study, high infection rates (69.4%) persisted even on farms practicing routine disinfection, and no significant difference was observed compared with farms where disinfection was not applied (Table 3). This supports previous findings that many commercial disinfectants are ineffective against oocysts at recommended concentrations. At the same time, higher doses may be toxic (24). The ineffectiveness likely results from inadequate cleaning before disinfection, as organic residues may protect oocysts within underlying layers (7).

Hygiene is a fundamental element in the control of *Cryptosporidium* infections (59). In order to strengthen the calf's immune system and reduce the risk of contamination, it is recommended to change bedding regularly, apply quicklime to dried floors, and minimize contact with contaminated feces (60).

It has been reported that calves kept under unhygienic conditions have a threefold higher infection rate (6). Poor hygiene creates favorable conditions for oocyst survival and increases the rate of infection (61).

The association between cleaning methods and disease risk has also been linked to the type of barn flooring (e.g., soil, concrete) (25). Changing bedding more than 12 times a year may increase the risk of disease, as personnel and equipment can act as fomites in spreading infections (62). Deep and dry straw bedding may provide protection by reducing contact with feces (24).

However, the existing literature and findings from this study indicate that while hygiene is essential, it is not sufficient on its own for effective infection control. Considering the environmental resilience of *C. parvum*, cleaning and disinfection practices must be supported by more comprehensive management strategies (63).

Environmental and housing conditions are key determinants of *Cryptosporidium* transmission (64). Because young calves are highly susceptible, grouping animals by age is recommended to limit contact with older, asymptomatic shedders (24,65). Studies on dam-calf contact have yielded inconsistent results- some report reduced risk with brief postnatal contact, while others found no effect (25,53,66). Contamination of calving areas remains a significant source of exposure within the first hours after birth (16), as supported by the detection of positivity in a 3-day-old calf in this study.

The influence of housing and flooring on *Cryptosporidium* infection remains controversial. Some studies have suggested that individual housing provides partial protection, whereas others reported no difference between individual and group systems (24, 40,48). Sharing pens may increase exposure (32). Concrete floors have been linked to lower prevalence than soil, though results are inconsistent (25,66,67). Similarly, bedding type shows variable effects-straw may elevate risk, while deep, dry straw or slatted floors could be protective (24,31,42). High humidity and poor drainage can prolong oocyst survival (68). In this study, no significant association was found between housing conditions and infection rates (Table 3), highlighting the need to evaluate these variables together with other management factors.

Due to an underdeveloped immune system, *C. parvum* infection occurs predominantly in calves younger than four weeks, with prevalence decreasing as age increases (69,70). The infection usually manifests as watery diarrhea between 1 and 4 weeks of age, and the development of acquired immunity with age enhances resistance (71). Oocyst shedding is most intense during the first week of life and declines after day 21 (72,73). In the present study, the highest prevalence (84%) occurred in calves aged 1-10 days, supporting the inverse relationship between age and susceptibility (53). The high infection rate in neonates likely increases environmental contamination, particularly in large herds where calf density is high, thereby perpetuating transmission (23,38). Moreover, *C. parvum* shedding by clinically healthy adult cattle can serve as an initial infection source for newborns (74). Detection of positivity in a 3-day-old calf in this study, together with the reported 2-3 day prepatent period (53), suggests that infection may occur within the first hours after birth (75).

Conclusion

In conclusion, the prevalence of *Cryptosporidium* spp. in neonatal calves in the province of Denizli was determined to be 68.4%. The infection rate decreased with age, was higher in female calves compared to males, and was found at a lower rate in calves that received hyperimmune serum (*E. coli*) immediately after birth. Furthermore, molecular methods demonstrated higher sensitivity compared to microscopic examination. *Cryptosporidium* spp. is a common pathogen responsible for diarrheal syndrome in neonatal calves. It causes significant economic losses globally in the livestock industry through calf mortality and treatment costs. The high prevalence emphasizes the need for improved biosecurity and farm management practices, as well as the widespread adoption of preventive strategies. Its zoonotic potential increases the risk to public health. In this context, animal and human health should be considered as a whole, and the "One Health" approach should be adopted. Although limited in geographic scope and sample size, this study helps fill a gap in national data. It provides a foundation for future epidemiological research. More comprehensive studies are needed to clarify the transmission dynamics and to develop effective control strategies.

Acknowledgements

This article is derived from the doctoral thesis of Dr. Zeynep Toprak Cınar, completed in the Department of Parasitology, Faculty of Veterinary Medicine, Aydın Adnan Menderes University. The authors gratefully acknowledge the valuable support of all faculty members of the Department of Parasitology. Special thanks are extended to Dr. Selin Hacilarlıoglu and Dr. Metin Pekagirbas for their contributions to the laboratory analyses, and to Dr. Ahmet Fatih Demirel (Department of Animal Science, Faculty of Agriculture, Van Yüzüncü Yıl University) for his assistance with statistical analysis. This study was supported by the Scientific Research Projects Unit of Aydın Adnan Menderes University under project number VTF-21023.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication and/or funding of this manuscript.

References

1. Mickelsen WD, Evermann JF. In utero infection responsible for abortion, stillbirth and weak calves in beef cows. Vet Clin North Am Food Anim Pract. 1994;10(1):1-14. DOI: 10.1016/S0749-0720(15)30586-7
2. Smith DR. Field disease diagnostic investigation of neonatal calf diarrhoea. Vet Clin North Am Food Anim Pract. 2012;28(3):465-481. DOI: 10.1016/j.cvfa.2012.07.010
3. Acha SJ, Kühn I, Jonsson P, Mbazima G, Katouli M, Möllby R. Studies on calf diarrhoea in Mozambique: prevalence of bacterial pathogens. Acta Vet Scand. 2004;45:27-36. DOI: 10.1186/1751-0147-45-27
4. Donovan GA, Dohoo IR, Montgomery DM, Bennett FL. Passive immunity and calf morbidity and mortality. Prev Vet Med. 1998;34:31-46. DOI: 10.1016/S0167-5877(97)00060-3
5. Reijnders M, Van Roosmalen M, Holtslag H, Arts S, Pel S, Dufé D, Kaashoek M, Vertenten G, Van Werven T, Sietsma S, Roy C, Herman

N. Efficacy of a novel subunit vaccine against *Cryptosporidium parvum* diarrhoea in neonatal calves. Animals. 2025;15:132. DOI: 10.3390/ani15020132
6. Ayele A, Seyoum Z, Leta S. *Cryptosporidium* infection in bovine calves: prevalence and risk factors in northwest Ethiopia. BMC Vet Res. 2018;14:105. DOI: 10.1186/s13104-018-3219-7
7. Fayer R. General biology. In: Fayer R, Xiao L, editors. Cryptosporidium and Cryptosporidiosis. 2nd ed. USA: CRC Press; 2008. 1-42 p.
8. Bulumulla S, Xiao L, Feng Y, Ash A, Aleri J, Ryan U, Barbosa AD. *Cryptosporidium* in cattle: assessing zoonotic risk. Curr Res Parasitol Vector Borne Dis. 2025;8:100279. DOI: 10.1016/j.crpvbd.2025.100279
9. Chalmers RM, Davies AP. Clinical cryptosporidiosis: a mini-review. Exp Parasitol. 2010;124(1):134-146. DOI: 10.1016/j.exppara.2009.02.003
10. Thompson RC, Olson ME, Zhu G, Enomoto S, Abrahamsen MS, Hijjawi NS. *Cryptosporidium* and cryptosporidiosis. Adv Parasitol. 2005;59:77-158. DOI: 10.1016/S0065-308X(05)59002-X
11. Osteras O, Gjestvang MS, Vatn S, Solverod L. Perinatal death in production animals in the Nordic countries: incidence and costs. Acta Vet Scand. 2007;49(1):14-21. DOI: 10.1186/1751-0147-49-S1-S14
12. Sahal M, Terzi OS, Ceylan E, Kara E. Calf diarrhoea and prevention methods. Lalahan Hayvancilik Arastirma Enstit Derg. 2018;58:41-49. [available at]
13. Kaduková M, Schreiberová A, Mudroň P, Tóthová C, Gomulec P, Štrkolcová G. *Cryptosporidium* infections in neonatal dairy calves. Microorganisms. 2024;12:1416. DOI: 10.3390/microorganisms12071416
14. Rivero MR, Vissio C, Feliziani C, De Angelo C, Touz MC, Tiranti K, Lombardelli JA, Duartez FJ, Curletto L. *Cryptosporidium* in Argentina: epidemiology and research advances in humans, animals and the environment in the 21st century. Front Microbiol. 2025;16:1592564. DOI: 10.3389/fmicb.2025.1592564
15. Martin SW, Meek AH, Willeberg P. Veterinary epidemiology: principles and methods. USA: Iowa State University Press; 1987. 343 p.
16. Del Coco VF, Cordoba MA, Basualdo JA. *Cryptosporidium* infection in calves in Buenos Aires, Argentina. Vet Parasitol. 2008;158:31-35. DOI: 10.1016/j.vetpar.2008.08.018
17. Ezzaty Mirhashemi M, Zintl A, Grant T, Lucy FE, Mulcahy G, De Waal T. Diagnostic techniques for *Cryptosporidium* in animals. Exp Parasitol. 2015;151-152:14-20. DOI: 10.1016/j.exppara.2015.01.018
18. Xiao L, Morgan UM, Limor J, Escalante A, Arrowood M, Shulaw W, Thompson RC, Fayer R, Lal AA. Genetic diversity within *Cryptosporidium parvum* and related *Cryptosporidium* species. Appl Environ Microbiol. 1999;65(8):3386-3391. DOI: 10.1128/AEM.65.8.3386-3391.1999
19. Bin Kabir MH, Ceylan O, Ceylan C, Shehata AA, Bando H, Essa MI, Xuan X, Sevinc F, Kato K. Genotypes and subtypes of *Cryptosporidium* in diarrheic calves, lambs, and goat kids from Turkey. Parasitol Int. 2020;79:102163. DOI: 10.1016/j.parint.2020.102163
20. Lichtmannsperger K, Harl J, Freudenthaler K, Hinney B, Wittek T, Joachim A. *Cryptosporidium parvum*, *Cryptosporidium ryanae* and *Cryptosporidium bovis* in Austrian calves. Parasitol Res. 2020;119:4291-4295. DOI: 10.1007/s00436-020-06928-5
21. Ranjbar R, Fattahi R. Prevalence of *Cryptosporidium* spp. in calves under one year old in Ilam County, Iran (2014-2015). Electron Physician. 2017;9(6):4631-4635. DOI: 10.19082/4631
22. Garber LP, Salman MD, Hurd HS, Keefe T, Schlater JL. Risk factors for *Cryptosporidium* infection in dairy calves. J Am Vet Med Assoc. 1994;205:86-91. [available at]
23. Hailu M, Asmare K, Gebremedhin EZ, Sheferaw D, Gizaw D, Di Marco V, Vitale M. *Cryptosporidium* and *Giardia* in dairy calves in Ethiopia. Parasite Epidemiol Control. 2020;10:e00155. DOI: 10.1016/j.parepi.2020.e00155
24. Brainard J, Hooper L, McFarlane S, Hammer CC, Hunter PR, Tyler K. Modifiable risk factors for *Cryptosporidium* in calves: a systematic review. Parasitol Res. 2020;119:3571-3584. DOI: 10.1007/s00436-020-06890-2
25. Trotz-Williams LA, Martin SW, Leslie KE, Duffield T, Nydam DV, Peregrine AS. Management practices associated with within-herd prevalence of *Cryptosporidium parvum* shedding on dairy farms in southern Ontario. Prev Vet Med. 2008;83(1):11-23. DOI: 10.1016/j.prevetmed.2007.03.001
26. Szonyi B, Bordonaro R, Wade SE, Mohammed HO. Seasonal variation and molecular epidemiology of *Cryptosporidium* infection in dairy cattle in the New York City watershed. Parasitol Res. 2010;107:317-325. DOI: 10.1007/s00436-010-1864-4
27. Mann ED, Sekla LH, Nayar GPS, Koschik C. Infection with *Cryptosporidium* spp. in humans and cattle in Manitoba. Can J Vet Res. 1986;50:174-178. [available at]
28. Wang R, Wang H, Sun Y, Zhang L, Jian F, Qi M, Ning C. Characteristics of *Cryptosporidium* transmission in preweaned dairy cattle in Henan, China. J Clin Microbiol. 2011;49:1077-1082. DOI: 10.1128/JCM.02194-10
29. Chandler AL, Hobbs CA, Mosley BS, Berry RJ, Canfield MA, Qi YP, Siega-Riz AM, Shaw GM. Neural tube defects and maternal micronutrients related to one-carbon metabolism. Birth Defects Res A Clin Mol Teratol. 2012;94(11):864-874. DOI: 10.1002/bdra.23068
30. Zhang K, Wu Y, Jing B, Xu C, Chen Y, Yu F, Wei Z, Zhang Y, Cui Z, Qi M, Zhang L. Seasonal monitoring of *Cryptosporidium* species and genetic diversity in neonatal calves on large-scale farms in Xinjiang, China. J Eukaryot Microbiol. 2022;69:e12878. DOI: 10.1111/jeu.12878
31. Szonyi B, Chang YF, Wade SE, Mohammed HO. Factors associated with risk of *Cryptosporidium parvum* infection in dairy calves. Am J Vet Res. 2012;73(1):76-85. DOI: 10.2460/ajvr.73.1.76
32. Imre M, Ilie M, Imre K, Darabuș G. Risk factors for *Cryptosporidium* in diarrheic pre-weaned calves. Proceedings of the XVII International Congress on Animal Hygiene, Košice, Slovakia; 2015.
33. Starkey SR, Zeigler PE, Wade SE, Schaaf SL, Mohammed HO. Factors associated with shedding of *Cryptosporidium parvum* versus *Cryptosporidium bovis* in dairy cattle in New York State. J Am Vet Med Assoc. 2007;229:1623-1626. DOI: 10.2460/javma.229.10.1623
34. Lee SH, VanBik D, Kim HY, Lee YR, Kim JW, Chae M, Oh SI, Goo YK, Kwon OD, Kwak D. Multilocus typing of *Cryptosporidium* spp. in diarrheic calves in Korea. Vet Parasitol. 2016;229:81-89. DOI: 10.1016/j.vetpar.2016.09.019
35. Garro CJ, Morici GE, Utges ME, Tomazic ML, Schnittger L. *Cryptosporidium* shedding in dairy calves in Argentina. Parasite Epidemiol Control. 2016;1:36-41. DOI: 10.1016/j.parepi.2016.03.008
36. Wells SJ, Garber LP, Hill GW. Health status of preweaned dairy heifers in the United States. Prev Vet Med. 1997;29:185-199. DOI: 10.1016/S0167-5877(96)01078-1
37. United States Department of Agriculture. Dairy 2007. Part II: changes in the US dairy cattle industry, 1991-2007. USA: USDA-APHIS-VS, CEAH; 2008. 57-61 p.
38. Naciri M, Paul Lefay M, Mancassola R, Poirier P, Chermette R. Role of *Cryptosporidium parvum* in neonatal diarrhoea complex in suckling and dairy calves in France. Vet Parasitol. 1999;85:245-257. DOI: 10.1016/S0304-4017(99)00111-9
39. Trotz-Williams LA, Martin SW, Leslie KE, Duffield T, Nydam DV, Peregrine AS. Calf-level risk factors for neonatal diarrhoea and shedding of *Cryptosporidium parvum* in Ontario dairy calves. Prev Vet Med. 2007;82(1-2):12-28. DOI: 10.1016/j.prevetmed.2007.05.003
40. Diaz P, Varcasia A, Pipia AP, Tamponi C, Sanna G, Prieto A, Ruiu A, Spissu P, Diez-Banos P, Morrondo P, Scala A. Molecular characterization and risk factors for *Cryptosporidium* spp. in calves from Italy. Parasitol Res. 2018;117(10):3081-3090. DOI: 10.1007/s00436-018-6000-x
41. Reimus K, Alvasen K, Emanuelson U, Viltrop A, Motus K. Herd-level risk factors for cow and calf on-farm mortality in Estonian dairy herds. Acta Vet Scand. 2020;62:15. DOI: 10.1186/s13028-020-0513-x
42. Urie NJ, Lombard JE, Shivley CB, Adams AE, Kopral CA, Santin M. Preweaned heifer management on US dairy operations: part III. Factors

associated with *Cryptosporidium* and *Giardia* in preweaned heifers. J Dairy Sci. 2018;101(10):9199-9213. DOI: 10.3168/jds.2017-14060
43. Harp JA, Goff JP. Strategies for control of *Cryptosporidium parvum* infection in calves. J Dairy Sci. 1998;81(1):289-294. DOI: 10.3168/jds.S0022-0302(98)75578-X
44. Huvepharma NV. Parofor Crypto 140 mg/ml Oral Solution for pre-ruminant calves. 2019. [available at]
45. Aydogdu U, Isik N, Ekici OD, Yildiz R, Sen I, Coskun A. Comparison of halofuginone lactate and paromomycin in calves naturally infected with *Cryptosporidium parvum*. Acta Sci Vet. 2018;46:1524. DOI: 10.22456/1679-9216.81809
46. Joachim A, Krull T, Schwarzkopf J, Daugschies A. Prevalence and control of bovine cryptosporidiosis in German dairy herds. Vet Parasitol. 2003;112:277-288. DOI: 10.1016/S0304-4017(03)00006-2
47. Naciri M, Mancassola R, Yvore P, Peeters JE. Effect of halofuginone lactate on experimental *Cryptosporidium parvum* infection in calves. Vet Parasitol. 1993;45:199-207. DOI: 10.1016/0304-4017(93)90075-X
48. Cruvine LB, Ayres H, Beltran Zapa DM, Nicaretta JE, Monteiro Couto LF, Heller LM, Bastos TSA, Cruz BC, Soares VE, Teixeira WF, De Oliveira JS, Fritzen JT, Alfieri AA, Freire RL, Zanetti Lopes WD. Diarrhea agents (*Coronavirus*, *Rotavirus*, *Cryptosporidium* spp., *Eimeria* spp., nematodes) in dairy calves by age. Trop Anim Health Prod. 2020;52:777-791. DOI: 10.1007/s11250-019-02069-9
49. House JK, Smith GF, McGuirk SM, Gunn AA, Izzo M. Disease in neonatal ruminants. In: Smith BP, editor. Large Animal Internal Medicine. 5th ed. USA: Elsevier; 2015. 302-338 p.
50. Matoock MY, El-Bably MA, El-Bahy MM. Management practices for minimizing environmental risk factors associated with *Cryptosporidium* in dairy calves. Vet Med J Giza. 2005;53(2):565-576. [available at]
51. Urie NJ, Lombard JE, Shivley CB, Kopral CA, Adams AE, Earleywine TJ, Olson JD, Garry FB. Preweaned heifer management on US dairy operations: part I. Descriptive characteristics. J Dairy Sci. 2018;101:1-17. DOI: 10.3168/jds.2017-14010
52. Thomson S, Hamilton CA, Hope JC, Katzer F, Mabbott NA, Morrison LJ, Innes EA. Bovine cryptosporidiosis: impact, host-parasite interaction and control strategies. Vet Res. 2017;48(1):42. DOI: 10.1186/s13567-017-0447-0
53. Silverlas C, Emanuel U, De Verdier K, Björkman C. Prevalence and management factors associated with *Cryptosporidium* shedding in Swedish dairy herds. Prev Vet Med. 2009;90(3-4):242-253. DOI: 10.1016/j.prevetmed.2009.04.006
54. Xiao L, Fayer R. Molecular characterisation of species and genotypes of *Cryptosporidium* and *Giardia* and assessment of zoonotic transmission. Int J Parasitol. 2008;38:1239-1255. DOI: 10.1016/j.ijpara.2008.03.006
55. Delafosse A, Chartier C, Dupuy MC, Dumoulin M, Pors I, Paraud C. Risk factors for *Cryptosporidium parvum* in dairy calves in western France. Prev Vet Med. 2015;118:406-412. DOI: 10.1016/j.prevetmed.2015.01.005
56. Mohammed HO, Wade SE, Schaaf S. Risk factors associated with *Cryptosporidium parvum* infection in dairy cattle in southeastern New York State. Vet Parasitol. 1999;83:1-13. DOI: 10.1016/S0304-4017(99)00032-1
57. McGuirk SM. Solving calf morbidity and mortality problems. In: Proceedings of the 36th Annual Conference of the American Association of Bovine Practitioners; 2003 Sep 15-17; Columbus, OH. Auburn (AL), USA: American Association of Bovine Practitioners; 2003. [available at]
58. Hammon HM, Frieten D, Gerbert C, Koch C, Dusel G, Weikard R, Kühn C. Milk diets affect jejunal mucosal immunity in pre-weaning calves. Sci Rep. 2018;8:1693. DOI: 10.1038/s41598-018-19954-2
59. Lorenz I, Fagan J, More SJ. Calf diarrhoea management in pre-weaned calves. Ir Vet J. 2011;64:9. DOI: 10.1186/2046-0481-64-9
60. Silverlas C, De Verdier K, Emanuelson U, Mattsson JG, Björkman C. *Cryptosporidium* infection in dairy herds with and without calf diarrhoeal problems. Parasitol Res. 2010;107:1435-1444. DOI: 10.1007/s00436-010-2020-x
61. El-Khodery SA, Osman SA. *Cryptosporidium* infection in buffalo calves. Trop Anim Health Prod. 2008;40:419-426. DOI: 10.1007/s11250-007-9113-2
62. Sischo WM, Atwill ER, Lanyon LE, George J. *Cryptosporidium* on dairy farms and contamination of surface water in the northeastern United States. Prev Vet Med. 2000;43(4):253-267. DOI: 10.1016/S0167-5877(99)00107-5
63. De Waele V, Speybroeck N, Berkvens D, Mulcahy G, Murphy TM. Control of calf cryptosporidiosis using halofuginone lactate. Prev Vet Med. 2010;96:143-151. DOI: 10.1016/j.prevetmed.2010.06.017
64. Kvac M, Vitovec J. Prevalence and pathogenicity of *Cryptosporidium andersoni* in beef cattle. J Vet Med B. 2003;50:451-457. DOI: 10.1046/j.0931-1793.2003.00701.x
65. Innes EA, Chalmers RM, Wells B, Pawlowic MC. One Health approach to cryptosporidiosis. Trends Parasitol. 2020;36(3):290-303. DOI: 10.1016/j.pt.2019.12.016
66. Maddox-Hyttel C, Langkjaer RB, Enemark HL, Vigre H. *Cryptosporidium* and *Giardia* in Danish cattle and pigs: occurrence and management factors. Vet Parasitol. 2006;141(1-2):48-59. DOI: 10.1016/j.vetpar.2006.04.032
67. Weber SE, Lippuner C, Corti S, Deplazes P, Hassig M. Clinical epidemiology of cryptosporidiosis in calves. Schweiz Arch Tierheilkd. 2016;158(5):341-350. DOI: 10.17236/sat00062
68. Barwick R, Mohammed H, White M, Bryant R. Prevalence of *Giardia* spp. and *Cryptosporidium* spp. on dairy farms in New York state. Prev Vet Med. 2003;59:1-11. DOI: 10.1016/S0167-5877(03)00052-7
69. Diaz P, Navarro E, Remesar S, Garcia-Dios D, Martinez-Calabuig N, Prieto A, Lorenzo GL, Manuel LC, Panadero R, Fernandez G, Diez-Banos P, Morrondo P. Age-related *Cryptosporidium* species in asymptomatic cattle in Spain. Animals. 2021;11:256. DOI: 10.3390/ani11020256
70. Santin M, Trout JM, Fayer R. Longitudinal study of cryptosporidiosis in dairy cattle from birth to two years of age. Vet Parasitol. 2008;155(1-2):15-23. DOI: 10.1016/j.vetpar.2008.04.018
71. Zhang Z, Su D, Meng X, Liang R, Wang W, Li N, Guo Y, Li S, Zhao Z, Xiao L, Feng Y. Cryptosporidiosis outbreak caused by *Cryptosporidium parvum* subtype IIdA20G1 in neonatal calves. Transbound Emerg Dis. 2021;00:1-8. DOI: 10.1111/tbed.13976
72. Sari B, Arslan MO, Gicik Y, Kara M, Tasci GT. Prevalence of *Cryptosporidium* spp. in diarrhoeic lambs in Kars province and associated risk factors. Trop Anim Health Prod. 2009;41:819-826. DOI: 10.1007/s11250-008-9260-0
73. Xiao L, Herd RP. Infection patterns of *Cryptosporidium* and *Giardia* in calves. Vet Parasitol. 1994;55:257-262. DOI: 10.1016/0304-4017(93)00645-F
74. Thomson S, Innes EA, Jonsson NN, Katzer F. Shedding of *Cryptosporidium* in calves and dams: evidence of reinfection and gp60 subtype diversity. Parasitol. 2019;146:1-10. DOI: 10.1017/S0031182019000829
75. Avendano C, Ramo A, Vergara-Castiblanco C, Sanchez-Acedo C, Quilez J. Genetic uniqueness of *Cryptosporidium parvum* from dairy calves in Colombia. Parasitol Res. 2018;117(5):1317-1323. DOI: 10.1007/s00436-018-5818-6

تأثير ممارسات إدارة المزارع على انتشار عدوى *Cryptosporidium* في العجول حديثي الولادة في مقاطعة دنيزلي، تركيا

زينب توبراك سينار[١]، عثمان سلجوق الديمير[٢]، سركان بكرسي[٢]

[١]مديرية دنيزلي للزراعة والغابات الإقليمية، وزارة الزراعة والغابات، دنيزلي، [٢] قسم علم الطفيليات، كلية الطب البيطري، جامعة عيد أوشن عدنان مندريس، عيد أوشن، تركيا

الخلاصة

تهدف هذه الدراسة إلى تقييم انتشار *Cryptosporidium* spp. في العجول حديثي الولادة وارتباطها بممارسات إدارة المزرعة. تم إجراء البحث في ٢٦ مزرعة ماشية تقع في مقاطعة دنيزلي، تيشركي، من خلال جمع عينات برازية من إجمالي ٢٥٠ عجلا حديثي الولادة. تم فحص العينات باستخدام صبغة كينيون السريعة الحمضية، متبوعا بطرق تفاعل البلمرة المتسلسل المتداخل، إذ تم تحديد انتشار *Cryptosporidium* spp. بـ ٣٨,٨٪ و ٦٨,٤ ٪ على التوالي. تم جمع بيانات إدارة المزرعة وتحليلها باستخدام اختبار كاي سكوير. تم العثور على معدل الإصابة ليكون أعلى في العجول التي تتراوح أعمارها بين ١-١٠ أيام ٨٤ ٪ وفي العجول الإناث ٨٧,٤٪. بالإضافة إلى ذلك ، لوحظ انخفاض معدل الإصابة بنسبة ٤٦,٢ ٪ في العجول التي تدار بمصل فرط المناعة عند الولادة. ونتيجة لذلك، أظهرت طرق تفاعل البلمرة المتسلسل المتداخل حساسية أعلى مقارنة بصبغة كينيون السريعة الحمضية. كما وجد أن انتشار *Cryptosporidium* spp. في العجول حديثي الولادة في دنيزلي كانت مرتفعة بشكل ملحوظ ٦٨,٤٪، وتسليط الضوء على ضرورة تحسين إدارة المزرعة. تنوع العوامل المرتبطة بالعدوى يعقد تخطيط الممارسات البيطرية الوقائية. نظرا للطبيعة الحيوانية المنشأ *Cryptosporidium*، يجب اعتماد استراتيجية "صحة واحدة"، وينبغي اتخاذ تدابير أكثر شمولا وتنسيقا بشكل مستدام للصحة العامة والأمن البيولوجي لتقليل معدلات الإصابة وحماية صحة الحيوان والبشر.