Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Comparative Immunology, Microbiology and Infectious Diseases 53 (2017) 50–55

Contents lists available at ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

journal homepage: www.elsevier.com/locate/cimid

Full Length Article

Effect of colostrum quantity and quality on neonatal calf diarrhoea due to *Cryptosporidium* spp. infection

K. Arsenopoulos[a], A. Theodoridis[b], E. Papadopoulos[a,*]

[a] *Laboratory of Parasitology and Parasitic Diseases, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, 541 24, Thessaloniki, Greece*

[b] *Laboratory of Animal Production Economics, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, 541 24, Thessaloniki, Greece*

ARTICLE INFO

Keywords:
Colostrum quality
Colostrum quantity
Cryptosporidium spp.
Neonatal calf diarrhoea
Season

ABSTRACT

This study was conducted to assess the effect of colostrum quality and quantity on *Cryptosporidium* spp. calf diarrhoea in an intensive dairy cattle farm in Greece. Faecal samples were collected from 100 dairy calves randomly selected and born during all 4 seasons (March 2015 to May 2016) of the year. In total, 71% of the selected calves were positive for *Cryptosporidium* spp. oocysts. The statistical analysis revealed influence of colostrum quality on faecal score. Linear regression showed that the colostrum quantity during the first day of life was negatively associated with the number of *Cryptosporidium* spp. oocysts in faeces. During multivariable analysis, the variables representing the quality of colostrum and the season of the calf's birth were identified as confounders. *Cryptosporidium* spp. is a common pathogen participating in neonatal calf diarrhoea. Colostrum management and season influence the number of *Cryprosporidium* spp. oocysts and faecal consistency. The above findings demonstrate novel risk factors that should be included in the strategic approaches to control cryptosporidiosis in newborn calves.

1. Introduction

Neonatal calf diarrhoea (NCD) is a syndrome of young calves aged less than 1 month old, provoked by the combination of infectious and non infectious causes [1]. Enterotoxigenic *Escherichia coli* (ETEC), rotaviruses, coronaviruses and *Cryptosporidium* spp. represent the four most prevalent infectious causes of calf diarrhoea, all over the world [2,3]. Two of them, *Cryptosporidium* spp. and rotaviruses, are the most commonly found causes in cases of diarrhoeic faecal samples [3,4]. In Greece, cryptosporidiosis plays an important role in NCD (among dairy cattle farms) and poses a significant public health issue [5].

Cryptosporidium spp. is a protozoan parasite, infecting a huge variety of mammals, birds, reptiles and fish. This protozoan has a direct life cycle and can be transmitted through the faecal-oral route, especially with food and water [6]. The first report of *Cryptosporidium* spp. infection in newborn calves causing diarrhoea was described in 1970 [7]. Since then, *Cryptosporidium* spp. has been considered as one of the most common pathogens present in calves during the first 2 weeks of their life [8,9].

Cryptosporidiosis in calves includes a non-specific diarrhoea accompanied with dehydration, anorexia as well as abdominal pain [10]. Clinical signs appear 3–5 days post infection and persist from 4 to 17 days [9]. If no action is taken, calf mortality is the most frequent outcome of *Cryptosporidium* spp. diarrheoa.

The clinical manifestations are influenced by many factors, which can be divided in three groups; factors associated with (a) calves, (b) environment and (c) production practices [11]. More specific, these include colostrum management, housing and hygiene, feeding, stress periods, season of the year, drugs administration and preventive measures of other immune modulating infectious diseases, such as Bovine Viral Diarrhoea (BVD), Infectious Bovine Rhinotracheitis (IBR) etc. [1].

Among all the above, colostrum management is the most important preventive measure, leading to adequate passive transfer to the newborn calves and thus to the reduction of the neonatal diarrheoa, including *Cryptosporidium* spp. [12,13]. However, there are some controversial reports supporting the lack of correlation between colostrum management and the risk of *Cryptosporidium* spp. diarrhoea [4,14]. The aim of this study was to assess the effect of colostrum quality (CQL) and quantity (CQT) on *Cryptosporidium* spp. diarrhoea during the different seasons of the year in an intensive dairy cattle farm in Greece.

* Corresponding author.
E-mail address: eliaspap@vet.auth.gr (E. Papadopoulos).

http://dx.doi.org/10.1016/j.cimid.2017.07.005
Received 10 April 2017; Received in revised form 3 July 2017; Accepted 4 July 2017

Fig. 1. Individually housing of neonatal calves.

2. Materials and methods

2.1. Herd history

The herd was consisted of 350 dairy cows of Holstein breed in lactation, reared under intensive management, in Thessaly (Greece). The animals consumed total mixed ratio (TMR) according to the nutrient requirements of their productive stage. The animals had a dry period of, at least, 2 months duration. During the last month of their dry period (9th month), they were routinely vaccinated against *Clostridium* spp. (Covexin®, Intervet) and rotavirus, coronavirus and *Escherichia coli* K99 (Rotavec Corona®, MSD). In addition, a vaccination against mastitis was applied in all animals (Startvac®, Hipra) of the farm.

2.2. Faecal sampling

The faecal sampling was carried out from a total of 100 neonatal calves, randomly selected, born during March 2015 to May 2016 (including all 4 seasons of the year, i.e. spring 2015 until end of spring 2016) and housed individually for the 2 month milk feeding period (Fig. 1). Individual faecal samples were collected directly from the rectum of each calf using sterile plastic gloves and stored in an isothermal box (approx. at 4 °C) before transfer to the Laboratory of Parasitology and Parasitic Diseases at the Faculty of Veterinary Medicine of Aristotle University of Thessaloniki. At the time of collection, the consistency (fecal score-FS) of each faecal sample was recorded according to the following manner: (1) normal (Fig. 2), (2) soft (Fig. 3) and (3) watery (Fig. 4) faeces. Sampling was done in accordance to animal welfare and did not cause any significant stress to the animals.

2.3. Faecal examination for Cryptosporidium spp. and other pathogens

Screening for *Cryptosporidium* spp. oocysts was performed by staining faecal smears according to Ziehl-Nielsen technique [15] and observing at 1000 x magnification under a phase contrast microscope. The intensity of infection for each faecal sample was evaluated semi-quantitatively according to the total number of oocysts per 10 randomly selected microscopic fields. In order to avoid any observation bias, only one person conducted all tests.

The possible presence of other agents of neonatal diarrhoea (rotavirus, coronavirus and *Escherichia coli*-K99) was detected using the diagnostic "Test kit Bovine Faeces (BIO 156)" (MSD).

Fig. 2. Normal faeces (FS 1) of calf.

Fig. 3. Soft faeces (FS 2) of calf.

2.4. Colostrum quality evaluation

CQL was evaluated for each cow (with a calf included in the study) directly after the parturition, with the help of a colostrum meter (Fig. 6). The colostrum meter was used according to the manufacturers' instructions. The CQL was categorized in three levels: (1) bad, as

Fig. 4. Watery faeces (FS 3) of calf.

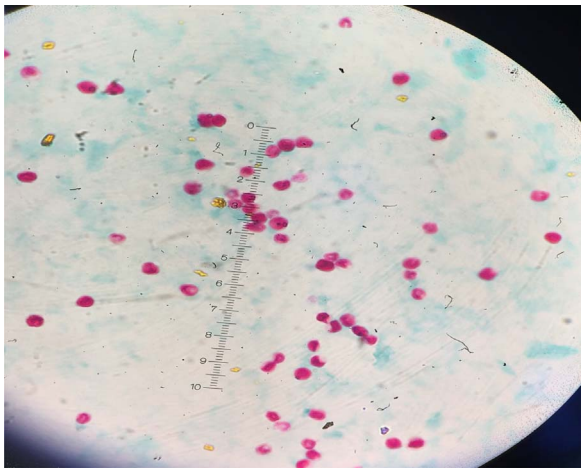

Fig. 5. *Cryptosporidium* spp. oocysts in faecal smears (Ziehl-Nielsen stain, 1000X).

indicated by the red color (< 20 g IgG/l, density 1025-1035), (2) middle, as indicated by the light green color (20–50 g IgG/l, density 1035-1045) and (3) good, as indicated by the green color (> 50 g IgG/l, density > 1045) colostrum.

2.5. Colostrum quantity measurement

The total volume of the two colostrum meals, fed during the first 24 h, was recorded using a graduated measuring cylinder. The first colostrum meal of each neonatal calf was given within the first 4 h of life.

2.6. Statistical analysis

Both parametric and non-parametric statistical methods were applied for the statistical evaluation of the data. The assumptions of normality and homogeneity of variances for the continuous variables were tested using the Shapiro-Wilk and Levene's test, respectively. In cases where the assumptions of variability and/or normality of the population's distribution were seriously violated, the Kruskal-Wallis non-parametric test was applied to evaluate group depended differences, while differences between median values of specific groups were evaluated using the non-parametric Wilcoxon rank sum test (Mann-Whitney *U* test). Moreover, the differences in proportions of the categorical variables between seasons were estimated through the application of a contingency table analysis (CQL and FS). Onwards, a simple linear regression model was employed to estimate the association between *Cryptosporidium* spp. number and CQT and a logistic regression model to evaluate the relationships between FS and potential risk factors. The specification chosen for the application of the logit approach was based on the statistical significance of the predictors. The FS was selected as the dependent variable (1 = normal, 2 = soft, 3 = watery), while CQL, CQT and season were selected as explanatory variables. A P value ≤ 0.05 was considered statistically significant. All analyses were conducted using the statistical software program SPSS (v. 23.0).

3. Results

3.1. In total results

In total, 71 of 100 (71%) calves were positive for *Cryptosporidium* spp. oocysts (Fig. 5). Regarding FS, the distribution, among positive faecal samples, was: 32 out of 71 (45%) calves had normal faeces (FS

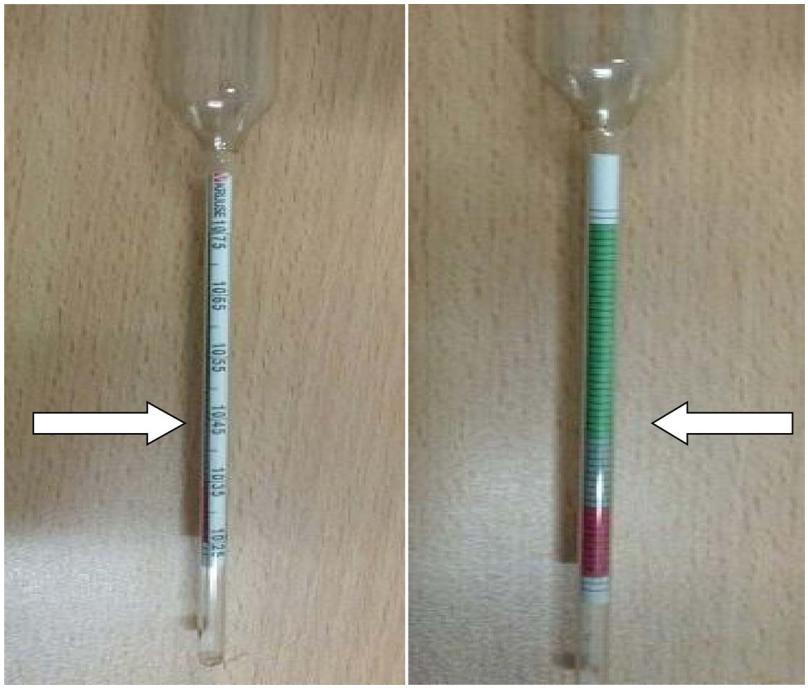

Fig. 6. Colostrum meter. The arrows indicate the colostrum density 1045 which consists the limit for an adequate passive transfer.

Table 1
Mean number (± SD) of *Cryptosporidium* spp. oocysts per faecal score (normal, soft and watery) of the calves (%).

Faecal Score	Mean number (± SD) of *Cryptosporidium* spp. oocysts
Normal n = 60 (60%)	3.93 [a] ± 4.92
Soft n = 19 (19%)	13.79 [b] ± 8.84
Watery n = 21 (21%)	35.67 [a,b] ± 22.11

[a, b] Different superscripts in the same column indicate statistical significant differences among faecal scores (P ≤ 0.05).

1), 18 out of 71 (25.4%) calves had soft faeces (FS 2) and the rest (21/71, 29.6%) had watery faeces (FS 3). Among *Cryptosporidium* spp. negative faecal samples, 28 out of 29 (96.6%) calves had normal faeces (FS 1), while one calf out of the 29 (3.4%) recorded as FS 2 (soft faeces). An overall significant (P ≤ 0.001) influence of *Cryptosporidium* spp. burden on the consistency of the examined faecal samples was recorded. The mean number (± SD) of *Cryptosporidium* spp. oocysts per fecal score was presented in Table 1. The diagnostic test kit results for rotavirus, coronavirus and *E. coli* (K99) were negative for the samples.

3.2. Effect and prediction model of colostrum quality on calf faecal consistency

CQL influenced significantly the faecal consistency (P ≤ 0.001). More precisely, the middle CQL affected all types of FS (normal, soft and watery faeces), while the rest (bad and good) influenced only the normal and soft FS (Table 2).

The effects of the studied risk factor (CQL), forced into the regression model, on FS are presented in Table 3. When neonatal calves were fed with bad CQL, the probability of watery faeces (FS 3) was 73.69% (0.55–0.92, P ≤ 0.001), of soft faeces (FS 2) was 21.03% (0.058–0.36, P ≤ 0.05) and of normal faecal consistency (FS 1) was 5.28% (0.002–0.103, P ≤ 0.05). When calves were fed with middle CQL, the probability of watery (FS 3), soft (FS 2) and normal faeces (FS 1) was 23.16% (0.072–0.391, P ≤ 0.05), 42.70% (0.286–0.568, P ≤ 0.001) and 34.14% (0.160–0.528, P ≤ 0.001), respectively. Finally, good CQL enhanced FS 1 (normal faecal consistency) (92.54%, 0.856–0.995, P ≤ 0.001) and reduced watery (FS 3) (1.24%, 0.001–0.026, P ≥ 0.05) and soft faecal consistency (FS 2) (6.21%, 0.003–0.122, P ≤ 0.05), respectively.

3.3. Effect of colostrum quantity on Cryptosporidium spp. burden

Linear regression showed that the CQT fed to the calves during the first day of their life was negatively associated (P ≤ 0.001) with the number of *Cryptosporidium* spp. oocysts shedding through faeces. The equation (Model 1), summarizing the effect of the CQT on *Cryptosporidium* spp. number, is the following:

Table 3
The marginal effects of colostrum quality on the faecal score of the experimental calves.

Colostrum Quality	Faecal Score	Marginal effects	P-value
Bad	Normal	5.28	0.040
	Soft	21.03	0.007
	Watery	73.69	0.000
Middle	Normal	34.14	0.000
	Soft	42.70	0.000
	Watery	23.16	0.004
Good	Normal	92.54	0.000
	Soft	6.21	0.041
	Watery	1.24	0.076

Cryptosporidium spp. number = 29.589 + (−3,258) colostum quantity (liters) (Model 1) $t_a = 5.134$ $t_b = -3.096$ (P ≤ 0.05, CI 95%, 98)

3.4. Interaction of the colostrum quality and season on Cryptosporidium spp. burden

During multivariable model analysis, the variables representing the CQL fed to calves in the first 24 h of the life, as well as the season of their birth were identified as confounders. These variables were forced into the model. Table 4 illustrates the results of *Cryptosporidium* spp. oocysts according to the interaction between CQL and season (P ≤ 0.001). The most interesting influence of the season on CQL and therefore, on *Cryptosporidium* spp. oocysts shedding, was during summer and winter.

3.5. Effect of season on faecal score

No effect of the season on the consistency of the calves' faeces (FS) were recorded according to our statistical analysis (P ≥ 0.05).

4. Discussion

The aim of this study was to assess the effect of CQL and CQT on *Cryptosporidium* spp. diarrhoea during the different seasons of the year in an intensive dairy cattle farm in Greece.

Till now, researchers have identified more than 20 species of *Cryptosporidium*, the majority of which are considered host specific and do not provoke clinical disease in immune competent hosts [6,16]. On the contrary, *Cryptosporidium parvum* (> 85% *Cryptosporidium* spp. infection) [1] can infect a wide variety of mammals, including cattle and has recognized as the main infectious cause of neonatal calf diarrhoea [6].

The prevalence of *Cryptosporidium* spp. shedding in our study was 71%. The prevalence found by Trotz-Williams et al. [4] and Delafosse et al. [9] are smaller than ours: 62% in Ontario dairy calves and 41.5% in Western France dairy calves, respectively. According to Meganck et al. [1], the prevalence of neonatal calf diarrhoea due to *C. parvum*

Table 2
Effect of colostrum quality on faecal score and the mean number (± SD) of *Cryptosporidium* spp. oocysts of the experimental calves (%).

Colostrum Quality	Faecal Score	Mean number (± SD) of *Cryptosporidium* spp. oocysts
Bad n = 18 (18%)	Normal n = 0 (0%)	0 [a]
	Soft n = 5 (27.8%)	11.2 [b] ± 4.32
	Watery n = 13 (72.2%)	40.8 [b] ± 21.24
Middle n = 29 (29%)	Normal n = 11 (37.9%)	1.92 [a] ± 2.61
	Soft n = 10 (34.5%)	15.1 [b] ± 5.68
	Watery n = 8 (27.6%)	27.37 [a,b] ± 22.28
Good n = 53 (53%)	Normal n = 49 (92.5%)	2.97 [a] ± 4.09
	Soft n = 4 (7.5%)	13.75 [b] ± 18.17
	Watery n = 0 (0%)	0 [b]

[a,b] Different superscripts in the same column indicate statistical significant differences among faecal scores (P ≤ 0.05).

Table 4
Effect of the interaction between colostrum quality and season on the mean number (SEM) of *Cryptosporidium* spp. Oocysts.

Season	Colostrum Quality		
	Bad	Medium	Good
Spring	25.00^{a}A	27.88^{a}A	3.00^{a}B
	−4.59	−3.98	−2.81
Summer	29.75^{ab}A	5.67^{b}B	0.67^{a}B
	−5.63	−6.5	−3.75
Autumn	10.50^{ab}A	6.78^{b}A	3.41^{a}A
	−7.96	−3.75	−2.73
Winter	49.33^{b}A	17.67^{ab}A	8.09^{a}B
	−4.59	−3.75	−3.4

[a, b] Different superscripts in each column indicate statistical difference of the mean number of *Cryptosporidium* spp. Oocysts among seasons ($P \leq 0.05$).
[A,B] Different uppercase letters in each row indicate statistical difference of the mean number of *Cryptosporidium* spp. Oocysts among colostrum quality ($P \leq 0.05$).

infection ranged between 27.8 and 63%. This difference can be explained by the fact that these countries implicate more modern management strategies than Greece. Previous studies conducted in Greece by Panousis et al. [5] reported a 25.05% prevalence of *Cryptosporidium* spp. among different aged (calves and cows) groups of animals and a 48.62% infection with *Cryptosporidium* spp. in young calves aged less than 14 days. Moreover, statistical analysis revealed that the number of *Cryptosporidium* spp. oocysts was strongly correlated with high percentage (55%) of diarrhoeic faeces (FS 2 and 3) among neonatal calves, leading to high percentages of morbidity and mortality. Torsein et al. [17] found that farms with high mortality among young calves have higher prevalence of *Cryptosporidium* spp. shedding than farms with low mortality. Therefore, it is confirmed that *Cryptosporidium* spp. remains a main enteropathogen.

The fact that one *Cryptosporidium* spp. negative calf had soft faeces confirms the presence of other causes, mainly infectious, of NCD that may co-infect or affect young calves leading to similar pathological outcome [2,3]. Therefore, other viral and bacterial pathogens of the gastrointestinal track such as *Escherichia coli,* rotavirus, coronavirus should be taken into consideration [9,10]. Calves born to vaccinated dams against those pathogens also shed less *C. parvum* oocysts [4]. The lower shedding of this protozoan (*C. parvum)* oocysts reflects a generally higher standard of management practices in these herds, rather than a direct protective effect. On the other hand, these enteropathogens can be also isolated in faecal samples from healthy calves, thus their presence may not be always the main cause of diarrhoea [3]. Nevertheless, all pregnant cows (one month before parturition) in our study were vaccinated against rotavirus, coronavirus and *Escherichia coli* K99 (Rotavec Corona®, MSD) in order to minimize any possible adverse effect of other pathogens than *Cryptosporidium* spp.

Colostrum management is one of the most significant preventive measures in order to reduce NCD, including *Cryptosporidium* spp. infection [18]. Particularly, both CQL and CQT fed in neonatal calves tend to be the first vital manipulations towards the improvement of newborn calves management [1].

The CQL is reflected by the number of immunoglobulins G (IgG) level. Therefore, colostral IgG concentration affects the status of passive immunity received by calves [19]. Unfortunately, IgG level in maternal colostrum varies dramatically among cows with a range of less than 1–235 g/L [20–22]. As a result, 29.4–57.8% of colostrum samples do not reach the proper level of 50 g IgG/L that characterizes the adequate passive immunity [21–23]. Moreover, the CQL cannot be estimated by the farmer based on produced volume or appearance of the colostrum. For all these reasons, colostral IgG content and thus, CQL is better to be measured with the aid of a colostrum meter (50 g IgG/l = density of 1045) or a brix refractometer (50 g IgG/l = 21-22 Brix) [24,25].

In our study, CQL influenced the number of *Cryptosporidium* spp. oocysts and therefore, the consistency of the examined faecal samples ($P \leq 0.001$). In the same frame, researchers support that adequate passive transfer due to good QL plays an important role to the prevention of neonatal calf diarrhoea [13,18]. On the contrary, Trotz-Williams et al. [4] found a non significant association between CQL and therefore, passive immunity and NCD. Windeyer et al. [26] concluded that neonatal calf diarrhoea was not statistically associated with failure of passive transfer to the calves.

CQT consists a critical point towards the prevention of NCD provoked by many causes, such as *Cryptosporidium* spp. This is because, the required amount of IgG level in the calves serum in order to achieve the maximum outcome is 150–200 g [27], thus, if colostrum contains 50 g IgG/L (good colostrum quality), feeding 4 L of colostrum are capable to provide 200 g IgG to the calf [1].

In our study, CQT fed to the calves was negatively related with the number of *Cryptosporidium* spp. oocysts shedding through faeces ($P \leq 0.001$) (Model 1). At the same time, the average amount of the consumed colostrum by the neonatal calves was 5.25 liters, at the first day of their life. It is obvious that the higher consumed colostrum, leading to adequate passive transfer, reduced the number of *Cryptosporidium* spp. oocysts in faecal samples. Unfortunately, only 8.9% of the standard deviation regarding *Cryptosporidium* spp. number can be interpreted due to the CQT consumed by neonatal calves. Thus, other factors additionally to CQT may influence *Cryptosporidium* spp. shedding through faeces. In accordance with our results, Trotz-Williams et al. [28] proposed that the early separation of calves from their dams and feeding an increased volume of colostrum within 6 h of birth is significantly associated with a reduced risk of failure of passive transfer [28] and thus, an enhanced immune system.

On the other hand, the CQT fed to calves during the first 24 h *post partum* was positively associated with the risk of *Cryptosporidium parvum* shedding [4]. According to Blaszkowska and Twardon [29] and Furman-Fratzcak et al. [13] calves fed with 1.3 L and 1.7 L of their first colostrum meal, respectively, developed adequate passive transfer, while individual ones with failure passive transfer consumed only 1.0 L of their first colostrum meal [29]. In a different study, Vasseur et al. [30] found that calves consumed 3.3 L of maternal colostrum during their first day of their life, while only 22% of them consumed less than 2 L (failure of passive transfer) [30]. The same authors concluded that the consumed CQT by the calf depends on the willingness to suck and thus, the relatively low amount observed in some calves was associated mainly with low vitality.

The season influenced the quality of the maternal colostrum and these two combined factors affected the average *Cryptosprodium* spp. shedding ($P \leq 0.001$), in our study. According to our findings, calves shed higher number of *Cryptosporidium* spp. oocysts during winter than summer. This result is in accordance with results conducted by Frank and Kaneene [31], Maddox-Hyttel et al. [32], Hamnes et al. [33], but opposite to the findings of Trotz-Williams et al. [4] who reported that oocysts shedding was positively associated with calf diarrhoea during summer months. Delafosse et al. [9] failed to demonstrate any significant association between season of birth and the number of *Cryptosporidum* spp. found in faecal samples. One possible explanation regarding our findings is that higher animal density, lower temperature and higher humidity during winter months increase the level of infectious agents (e.g. *Cryptosporidium* spp.) leading to high risk of diarrhoea [14,34].

Furthermore, Gulliksen et al. [20] found that season negatively influenced the CQL. More precisely, Norwegian dairy cows, calving during winter, produce a poorer CQL than during any other season of the year [20]. The importance of adequate consumption of colostrum by calves (at least 4 Liters of colostrum during the first 12 hours of their life) and its effect on the health of the calf is well established. Consequently, intake of colostrum with a lower concentration of IgG (bad quality) could further increase the risk of diarrhoea in calves born during this season.

Finally, our study highlights the significance of the CQL, given to neonatal calves in different quantities during the year, on the clinical appearance of cryptosporidiosis. This effect was found to vary greatly during the various seasons depending on the CQL. The results of this study confirm the beneficial value of the colostrum, as for the first time it was attempted to evaluate it applying measurements of its quality and quantity as risk factors for calf cryptosporidiosis.

5. Conclusions

The results of this study show that *Cryptosporidium* spp. is a common protozoan parasite in young dairy calves. The presence of this intestinal parasite is associated with high risk of diarrhoea and increased risk of calf mortality. Our study proved that risk factors such as the CQL and CQT, as well as the season, can affect the *Cryptosporidium* spp. shedding and the consistency of the faeces. The above findings demonstrate novel risk factors that should be included in the strategic approaches to control cryptosporidiosis.

Conflict of interest

None of the authors has any financial or personal relationships that could inappropriately influence the content of this paper.

References

[1] V. Meganck, G. Hoflack, G. Opsomer, Advances in prevention and therapy of neonatal dairy calf diarrhoea: a systematical review with emphasis on colostrum management and fluid therapy, Acta Vet. Scand. 56 (2014) 75.

[2] C.J.M. Bartels, M. Holzhauer, R. Jorritsma, W.A.J.M. Swart, T.J.G.M. Lam, Prevalence, prediction and risk factors of enteropathogens in normal and non-normal faeces of young Dutch dairy calves, Prev. Vet. Med. 93 (2010) 162–169.

[3] C. Silverlas, K. de Verdier, U. Emanuelson, J.G. Mattsson, C. Bjorkman, *Cryptosporidium* infection in herds with and without calf diarrhoeal problems, Parasitol. Res. 107 (2010) 1435–1444.

[4] L.A. Trotz-Williams, S.W. Martin, K.E. Leslie, T. Duffield, D.V. Nydam, A.S. Peregrine, Calf-level risk factors for neonatal diarrhoea and shedding of *Cryptosporidium parvum* in Ontario dairy calves, Prev. Vet. Med. 82 (2007) 12–28.

[5] N. Panousis, A. Diakou, E. Papadopoulos, N. Giadinis, H. Karatzias, S. Haralampidis, Prevalence of *Cryptosporidium* infection in dairy farms with a history of calves' diarrhoea, C. Pract. 15 (2007) 89–92.

[6] L. Xiao, Molecular epidemiology of cryptosporidiosis: an update, Exp. Parasitol. 124 (2010) 80–89.

[7] J. Pohlenz, H.W. Moon, N.F. Cheville, Cryptosporidiosis as a probable factor in neonatal diarrhoea of calves, J. Am. Vet. Med. Assoc. 172 (1978) 452–457.

[8] H.P. Thompson, J.S.G. Dooley, J. Kenny, M. McCoy, C.J. Lowery, J.E. Moore, L. Xiao, Genotypes and subtypes of *Cryptosporidium* spp. in neonatal calves in Northern Ireland, Parasitol. Res. 100 (2007) 619–624.

[9] A. Delafosse, C. Chartier, M.C. Dupuy, M. Dumoulin, I. Pors, C. Paraud, *Cryptosporidium parvum* infection and associated risk factors in dairy calves in western France, Prev. Vet. Med. 118 (2015) 406–412.

[10] D.C. de Graaf, E. Vanopdenbosch, L.M. Ortega-Mora, H. Abbassi, J.E. Peeters, A review of the importance of cryptosporidiosis in farm animals, Int. J. Parasitol. 29 (1999) 1269–1287.

[11] M.E. Olson, R.M. O' Handley, B.J. Ralston, T.A. McAllister, R.C. Thompson, Update on *Cryptosporidium* and *Giardia* infections in cattle, Trends Parasitol. 20 (2004) 185–191.

[12] A. Koch, M. Kaske, Clinical efficacy of intravenous hypertonic saline solution or hypertonic bicarbonate solution in the treatment of inappetent calves with neonatal diarrhoea, J. Vet. Intern. Med. 22 (2008) 202–211.

[13] K. Furman-Fratczak, A. Rzasa, T. Stefaniak, The influence of colostral immunoglobulin concentration in heifer calves' serum on their health and growth, J. Dairy Sci. 94 (2011) 5536–5543.

[14] S.M. Gulliksen, E. Jor, K.I. Lie, I.S. Hamnes, T. Loken, J. Akerstedt, O. Osteras, Enteropathogens and risk factors for diarrhoea in Norwegian dairy calves, J. Dairy Sci. 93 (2009) 5057–5066.

[15] S.A. Henriksen, J.F. Pohlenz, Staining of cryptosporidia by a modified Ziehl-Neelsen technique, Act. Vet. Scand. 22 (1981) 594–596.

[16] T. de Waal, Advances in diagnosis of protozoan diseases, Vet. Parasitol. 189 (2012) 65–74.

[17] M. Torsein, A. Lindberg, C.H. Dandgren, K.P. Waller, M. Tornquist, C. Svens-son, Risk factors for calf mortality in large Swedish dairy herds, Prev. Vet. Med. 99 (2011) 136–147.

[18] A.C.B. Berge, D.A. Moore, T.E. Besser, W.M. Sischo, Targeting therapy to minimize antimicrobial use in preweaned calves: effects on health, growth, and treatment costs, J. Dairy Sci. 92 (2009) 4707–4714.

[19] S.M. Godden, D.J. Smolenski, M. Donahue, J.M. Oakes, R. Bey, S. Wells, S. Sreevatsan, J. Stabel, J. Fetrow, Heat-treated colostrum and reduced morbidity in preweaned dairy calves: results of a randomized trial and examination of mechanisms of effectiveness, J. Dairy Sci. 95 (2012) 4029–4040.

[20] S.M. Gulliksen, K.I. Lie, L. Solverod, O. Osteras, Risk factors associated with colostrum quality in Norwegian dairy cows, J. Dairy Sci. 91 (2008) 704–712.

[21] M. Chigerwe, J.W. Tyler, M.K. Summers, J.R. Middleton, L.G. Schultz, D.W. Nagy, Evaluation of factors affecting serum IgG concentrations in bottle-fed calves, J. Am. Vet. Med. Assoc. 234 (2009) 785–789.

[22] K.M. Morrill, E. Conrad, A. Lago, J. Campbell, J.D. Quigley, H. Tyler, Nationwide evaluation of quality and composition of colostrum on dairy farms in the United States, J. Dairy Sci. 95 (2012) 3997–4005.

[23] H. Swan, S. Godden, R. Bey, S. Wells, J. Fetrow, H. Chester-Jones, Passive transfer of immunoglobulin G and preweaning health in Holstein calves fed a commercial colostrums replacer, J. Dairy Sci. 90 (2007) 3857–3866.

[24] V. Bielmann, J. Gillan, N.R. Perkins, A.L. Skidmore, S. Godden, K.E. Leslie, An evaluation of Brix refractometry instruments for measurement of colostrum quality in dairy cattle, J. Dairy Sci. 93 (2010) 3713–3721.

[25] J.D. Quigley, A. Lago, C. Chapman, P. Erickson, J. Polo, Evaluation of the Brix refractometer to estimate immunoglobulin G concentration in bovine colostrums, J. Dairy Sci. 96 (2013) 1148–1155.

[26] M.C. Windeyer, K.E. Leslie, S.M. Godden, D.C. Hodgins, K.D. Lissemore, S.J. LeBlanc, Factors associated with morbidity mortality, and growth of dairy heifer calves up to 3 months of age, Prev. Vet. Med. 113 (2014) 231–240.

[27] M. Chigerwe, J.W. Tyler, L. Schultz, J.R. Middleton, B.J. Steevens, J.N. Spain, Effect of colostrum administration by use of oroesophageal intubation on serum IgG concentrations in Holstein bull calves, Am. J. Vet. Res. 69 (2008) 1158–1163.

[28] L.A. Trotz-Williams, S.W. Martin, K.E. Leslie, T. Duffield, D.V. Nydam, A.S. Peregrine, Association between management practices and within-herd prevalence of *Cryptosporidium parvum* shedding on dairy farms in southern Ontario, Prev. Vet. Med. 83 (2008) 11–23.

[29] M. Blaszkowska, J. Twardon, Level of IgGg, IgG2 and IgM in colostrum and calves serum obtained during different periods following birth, Med. Welt. 61 (2005) 1308–1311 (in Polish).

[30] E. Vasseur, J. Rushen, A.M. de Passille, Does a calf's motivation to ingest colostrum depend on time since birth calf vigor, or provision of heat? J. Dairy Sci. 92 (2009) 3915–3921.

[31] N.A. Frank, J.B. Kaneene, Management risk factors associated with calf diarrhoea in Michigan dairy herds, J. Dairy Sci. 76 (1993) 1313–1323.

[32] C. Maddox-Hyttel, R.B. Langkjaer, H.L. Enemark, H. Vigre, *Cryptosporidium* and *Giardia* in different age groups of Danish cattle and pigs-occurence and mangement associated risk factors, Vet. Parasitol. 141 (2006) 48–59.

[33] I.S. Hamnes, B. Gjerde, L. Robertson, Prevalence of *Giardia* and *Cryptosporidium* in dairy calves in three areas of Norway, Vet. Parasitol. 140 (2006) 204–216.

[34] R. Fayer, M. Santin, L. Xiao, *Cryptosporidium bovis* n. sp. (Apicomplexa: Cryptosporidiidae) in cattle (*Bos taurus*), J. Parasitol. 91 (2005) 624–629.