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ELISA–based Point Prevalence of enteropathogens in diarrheic calves in Central Anatolia Region of Turkey

Prevalencia puntual de enteropatógenos basada en ELISA en terneros diarreicos en la región de Anatolia Central de Turquía

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ABSTRACT

This study reports potential causes of diarrhea in neonatal calves, leading to calf mortality, from the selected population of the three Provinces of Turkey. A total of 300 fecal samples were collected purposively from diarrheic neonatal calves distributed to the three age groups (1–14 days, 15–29 days, and 30–90 days), from Konya, Karaman, and Aksaray Provinces of Turkey. The fecal specimens were examined for the existence of *Cryptosporidium* spp., rotavirus, coronavirus, and *Escherichia coli* by commercially available capture direct enzyme linked immunosorbent assay (ELISA) kit. The oocysts and coproantigens of *Cryptosporidium* were identified in 109 (36.3%) and 156 (52%) of the 300 calves, respectively. While, rotavirus, *E. coli* and coronavirus antigens were detected ($P<0.05$) in 57 (19%), 17 (5.6%) and 6 (2%) calves, respectively. Mixed infection of the study pathogens has also been found in this report. These results provide a baseline information on the frequent causes of neonatal calf diarrhea in the studied Provinces which can be used to develop a prophylaxis plan.

Key words: *Cryptosporidium* spp.; coronavirus; rotavirus; *Escherichia coli*; diarrheic calves

RESUMEN

Este estudio informa sobre las posibles causas de diarrea en terneros recién nacidos, que conducen a la mortalidad de los terneros, de la población seleccionada de las tres provincias de Turquía. Se recolectaron intencionalmente un total de 300 muestras fecales de terneros recién nacidos con diarrea distribuidos en tres grupos según la edad (1–14 días; 15–29 días y 30–90 días), de las provincias de Konya, Karaman y Aksaray de Turquía. Las muestras fecales se examinaron para detectar la existencia de *Cryptosporidium* spp., rotavirus, coronavirus y *Escherichia coli* mediante un kit de ensayo inmunoabsorbente ligado a enzimas (ELISA) de captura directa disponible en el mercado. Los ooquistes y coproantígenos de *Cryptosporidium* se identificaron en 109 (36,3 %) y 156 (52 %) de los 300 terneros, respectivamente. Mientras que se detectaron antígenos de rotavirus, *E. coli* y coronavirus ($P<0,05$) en 57 (19 %), 17 (5,6 %) y 6 (2 %) terneros, respectivamente. En este informe también se ha encontrado una infección mixta de los patógenos del estudio. Estos resultados brindan información de referencia sobre las causas frecuentes de diarrea neonatal en terneros en las Provincias de estudio que pueden utilizarse para desarrollar un plan de profilaxis.

Palabras clave: *Cryptosporidium* spp.; coronavirus; rotavirus; *Escherichia coli*; becerros diarreicos

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INTRODUCTION

Enteric infections involving diarrhea, dehydration, and weight loss are considered to major health problems throughout the World and are significant issues on dairy farms [1]. The diarrhea becomes worse if the host susceptibility is compromised, e.g. in neonatal period, which includes the first 28 days following birth, and is refered as the neonatal calf diarrhea (NCD) [2]. The predominant causative agents of the NCD include: bacterial (*Escherichia coli* K99, *Clostridium* spp., *Salmonella* spp. and *Campylobacter* spp.), parasitic (*Cryptosporidium* spp. and *Coccidia* spp.), and viral (rotavirus and coronavirus) agents [2, 3]. Immunological conditions (vaccination and receiving quality of colostrum), management factors (environmental and hygienic conditions) and nutritional factors (feeding of adequate amounts of milk) also play a role in predisposing the animals towards the NCD [1].

Cryptosporidiosis has been reported as the cause of serious problems in many types of animals such as calves (*Bos taurus*), lambs (*Ovis aries*), and kids in the neonatal period. In particular, 1–3–week–old calves are highly susceptible to the disease, as it has been reported that oocysts can be found in calves as young as 4 days old, with severe diarrhea after three days of ingesting oocysts, and lasting for 2–14 days [4, 5].

Neonatal period has also been identified at risk for rotavirus infections [6, 7, 8]. Cattle of all ages are sensitive to rotavirus infections; however, higher prevalence has been reported in calves under one year [9]. Clinical findings of rotavirus infection are like to those of cryptosporidiosis [10]. Rotavirus infection involves only the small intestine; however, coronavirus infection involves both the small and large intestines and can lead to more severe outcomes and higher mortality rates [11].

E. coli is normal intestinal flora of humans and animals having various serotypes with different pathogenic courses, depending on the animal species [12]. The *E. coli* K99 strain is the most well–known enteropathogen in calves [10]. NCD *E. coli*–caused can be fatal due to rapid and excess loss of water and electrolytes from the body leading to severe weakness and hypothermia [13].

In this study, the aim was to identify the prevalent etiological agents causing NCD in Konya, Karaman, and Aksaray Regions of Turkey through Enzyme– linked immunosorbent assay (ELISA) for coproantigen detection.

MATERIALS AND METHODS

Fecal samples

Fecal samples were collected from 300 diarrheic calves aged 0–3 months (159 calves were aged between 0–14 days, 85 calves were aged between 15–29 days, 56 calves were aged between 30–90 days) from farms in three different Provinces of Turkey (Konya, Karaman, and Aksaray) between January 2014 and April 2014. The farms included in the study had similar climatic conditions. All calves were fed with colostrum after birth. Almost 20–30 grams (g) fecal samples were taken from the rectum in 5 mL eppendorf tube (Eppendorf Co., Germany) and kept in refrigerators (ThermoFisher Scientific, U.S.A.) at 4°C until tested.

Modified Ziehl–Neilsen (MZN) for *Cryptosporidium* oocysts

Fecal smears were made as per the standard protocols [14]. Briefly, fecal samples were mixed with 0.09% NaCl solution, spread thinly on the glass slide and dried at room temperature followed by modified Ziehl–Neilsen (MZN) stain to detect *Cryptosporidium* oocysts in the fecal smears under microscope (BX43 Olympus, Japan) with oil immersion lense (X100) [14].

The evaluation considered the number of oocysts in 20 randomly selected microscope fields in the preparations. The severity of the infection according to the *Cryptosporidium* oocyst density were defined as negative (no oocyst observed), mild (1–5 oocyst), moderate (6–10 oocyst) and severe (>10 oocyst) [15].

ELISA for coproantigen detection

A commercial ELISA kit (Bio–X Diagnostics, Belgium) was used to detect the presence of antigens against *Cryptosporidium* spp., coronavirus, rotavirus and *E. coli* in feces from all 300 diarrheic calves. Feces diluted in dilution buffer and incubated on the microplate for 1 h at 4°C. After incubation step, the plate washed and incubated for 1 h with the conjugate room tempurature, a peroxidase labelled antigen (*Cryptosporidium* spp., coronavirus, rotavirus and *E. coli*) specific monoclonal antibody. After this second incubation, the plate is washed again and the tetramethylbenzidine added and incubated 10 min in room temperature. The enzyme substrate reaction stopped with a stop solution and the results evaluated in a 450 nm ELISA reader.

Statistical analysis

The results of the research were evaluated by chi–square test (SPSS 2.0). $P<0.05$ value statistic was considered significant.

RESULTS AND DISCUSSION

Calves screened during the study were classified into three categories viz; 0–14 days (n=159), 15–29 days (n=85) and 30–90 days (n=56). The most common mixed infection was caused by the two agents viz; *Cryptosporidium* spp. and *Rotavirus* in 8% of the studied calves followed by *Coronavirus–E. coli* (0.3%) and *Cryptosporidium* spp.–*Rotavirus–E. coli* (0.6%) mixed infections. Mixed infection also followed the similar pattern of age wide distribution as did the *Cryptosporidium* being highest (12.9%) in 15–29 days aged calves followed in order by those aged 0–14 days (7.5%) and 30–90 days (1.7%). The only Coronavirus–*E. coli* mixed infection was observed in the 30–90 days age group; and *Cryptosporidium* spp.–Rotavirus–*E. coli* mixed infection were detected in 0–14 days (0.6%) and 15–29 days (1.1%) age groups.

This study found that *Cryptosporidium*, rotavirus, coronavirus, and *E. coli* were the enteropathogens involved in the etiology of NCD, and that *Cryptosporidium* and rotavirus were the most important enteropathogens, among these. It is also suggested that for NCD, it should be determined if the infection is of mixed nature as the clinical indications only are not sufficient to provide information about more than one pathogen involved. Furthermore, the diagnosis should be promoted by different diagnostic techniques [16]. For pathogen identification, staining methods such as: the MZN, Trichrome, Acridine Orange, Modifiye Köster and Kinyoun acid–fast stains (for *Cryptosporidium*) were used. Tests like the fluorescent antibody test and ELISA techniques (for rotavirus and and coronavirus), and bacteriological cultures for *E. coli*, are used [4, 17, 18]. The distribution of enteropathogens identified through commercial ELISA according to the age groups in the neonatal diarrheal calves are demonstrated in TABLE I. Among these, *Cryptosporidium* spp. and coronavirus were detected at the highest and lowest rates,

respectively. Rotavirus and *E. coli* were found to cause a moderate number of infections compared to *Cryptosporidium* spp. in calves with diarrhea in these regions. In this study, rotavirus was the second most common agent detected in 19% of diarrheic calves, while the overall prevalence of *E. coli* was 5.6% in diarrheic calves. The lowest infection rate detected was for coronavirus (2%). Age wide distribution of coronavirus was found to be 2.5 and 3.5% in 0–14– and 30–90–days groups, respectively; whereas, no coronavirus antigens were found in calves (n=85) of 15–29 days group (TABLE I).

TABLE I
Prevalence (%) of enteropathogens in the different age groups of diarrheic calves

Age (days)	0 –14	15–29	30–90	Total
Number of animals (n)	159	85	56	300
Cryptosporidium spp.	84 (52.8%)^{ab}	50 (58.8%)^{a}	22 (39.2%)^{b}	156 (52%) ^{A}
Rotavirus	20 (12.5%)^{a}	34 (40%)^{b}	7 (12.5%)^{a}	61 (19%)^{B}
Coronavirus	4 (2.5%)^{a}	0 (0.0%)^{a}	2 (3.5%)^{a}	6 (2%)^{D}
E. coli	8 (5%)^{a}	4 (4.7%)^{a}	5 (8.9%)^{a}	17 (5.6%)^{C}
Cryptosporidium spp., Rotavirus	12 (7.5%)^{ab}	11 (12.9%)^{a}	1 (1.7%)^{b}	24 (8%)^{C}
Coronavirus, *E. coli*	0 (0.0%)^{a}	0 (0.0%)^{a}	1 (1.7%)^{a}	1 (0.3%)^{D}
Cryptosporidium spp., Rotavirus, *E. coli*	1 (0.6%)^{a}	1 1.1%)^{a}	0 (0.0%)^{a}	2 0.6%)^{D}

Different letters in the same line (^{A,B,C,D}) and columns (^{a,b}) are statistically significant (Chi–square test, $P<0.05$)

The prevalence of cryptosporidiosis diagnosed by MZN and commercial ELISA in diarrheal stool specimens was 36.3 and 52%, respectively. Microscopic fecal examination of MZN–stained smears revealed *Cryptosporidium* spp. oocysts in 109 (36.3%) cases. The highest distribution (47%) was in 15–29 days age group followed in order by 0–14 days (33.9%) and 30–90 days (26.7%) age groups of calves. While the highest prevalence in this study was found in the calves aged 15–29 days, the differences between the age groups was statistically significant ($P<0.05$)(TABLE II). It was determined that 43% of the sick animals were severely infected (>10 oocyst)(TABLE III). All acid–fast positive samples showed a positive reaction on the ELISA.

TABLE II
Prevalence (%) of *Cryptosporidium* spp. in the different age groups of diarrheic calves by MZN

Age	Number of animals (n)	*Cryptosporidium* spp.	Infection rate (%)
0 –14 day	159	54	33.9^{b}
15–29 day	85	40	47^{a}
30–90 day	56	15	26.7^{b}
Total	300	109	36.3

Different letters in the same columns (^{a,b}) are statistically significant (Chi–square test, $P<0.05$)

TABLE III
Infection score according to age groups in calves infected with *Cryptosporidium* spp.

Amount of oocysts	0–14 days	15–29 days	30–90 days	Total
Negative (no oocyst)	105	45	41	191 (does not apply)
Mild (1–5 oocysts)	12	11	3	26 (23.85%)
Moderate (6–10 oocysts)	18	10	8	36 (33.02%)
Severe (>10 oocysts)	24	19	4	47 (43.11%)

Although management factors (e.g., environmental, and nutritional factors) for calves with concurrent infections of *Cryptosporidium* spp. and other agents may affect the outcome of the disease, the results of this research demonstrate the significance of *Cryptosporidium* spp. as the major pathogen responsible for acute diarrhea in neonatal calves. Regarding the most commonly detected pathogen of NCD, variable reports are present with rotavirus [19] and *Cryptosporidium* spp. as the most prevalent agent [20]. In Turkey, prevalence of *Cryptosporidium* spp. has been found 3.9–70.3% [21, 22, 23, 24]. In other Countries, prevalence reports range from 3.1 to 86.4% [25, 26, 27, 28, 29]. Risks factors like age and immune status are significant host–related determinants affecting the prevalence of cryptosporidiosis. The prevalence of *Cryptosporidium* spp. as determined through shedding of *Cryptosporidium* oocysts in different age groups is variable [30].

In this study, the severity of the infection according to the *Cryptosporidium* oocyst density was detected as 43%. Infection is often restricted to younger animals of 8 weeks or less and is most prevalent in calves that are less than one month of age [31]. The highest infection rates in this study were in calves of 15–29 days of age which is different from other reports where calves aged 1–10 [15], 7–14 [32], 8–14 [22], and 8–21 [33] days had been found most susceptible. The main reason for this was thought to be due to sudden antibody changes in their immune systems in case the calves received insufficient colostrum due to national and seasonal differences. In addition, rotavirus infection was found to be considerably positive in animals in this age group. It is thought that the surface of the intestinal mucosa may be colonized by *Cryptosporidium* oocysts due to the destruction of the intestinal mucosa after rotavirus infection. Although there are many vaccine studies against Cryptosporidiosis, the lack of an effective commercial vaccine is another reason for *Cryptosporidium* the high rate in NCD [34].

In the present study, rotavirus was found in 20.3% of the diarrheic calves which falls within the range (10.4–53%) of infections reported elsewhere in Turkey [35, 36, 37, 38]. Although the prevalence of rotavirus infection varies depending on factors such as the frequency of farm hygiene and protection and control measures, the detection of the disease in one of every five calves in the study group in this region indicates that the NCD disease is a disease that requires precautions. The distribution of rotavirus found in this research (20.3%) was comparable with those reported in diarrheic calves of Belgium (20%), Switzerland (59%), Netherlands (17.7%), and the Brazil (6,37%) [19, 31, 39, 40]. The prevalence of rotavirus was found highest in 15–29 days age–group calves in this study which is in agreement with the report of Mukhtar *et al.* from Pakistan [41].

E. coli is another significant NCD causing agent [17]. Previous studies in Turkey found that *E. coli* was detected in 9.4% of 192 diarrheal calves in East and Southeast of Turkey [33], 26% of 138 diarrheal calves in Sivas [42], and 24.81% of 133 diarrheal calves in part of eastern Turkey [43]. The prevalence of *E. coli* in NCD cases in other Countries has been reported as 0.9–56.3% [20, 44, 45]. Differences in findings may be due to disparities in the methods used or in the age groups studied, as well as regional differences in *E. coli* serotypes. Factors like shorter shedding period of bacteria may influence the distribution of *E. coli* [31]. In addition, the low rate of infection compared to other regions and studies indicates that the farms in these 4 regions apply effective protection control methods such as vaccination during pregnancy and hyperimmune sera administration to newborn calf against *E. coli* K99.

It has been stated that calves are quite sensitive to coronavirus during their first 3–21 days [46]. A yellowish, watery diarrhea, sometimes with the presence of mucous but rarely blood, develops and lasts for 3–6 days. Virus can be detected in the stool during this period [47]. In many former studies in Turkey, the prevalence of coronavirus infection in calves was reported between one and 37.1% [37, 38, 48, 49]. Hasoksuz *et al.* reported infection rates in 37.1, 25.6 and 18.2% of the cows in the age groups 0–30 days, 4–12 months, and 2–7 years, respectively [49]. In other Countries, prevalence of coronavirus in diarrheic calves has been found between 1.9 and 8% [28, 31, 39, 40]. Lower coronavirus prevalence may be associated with the previous use of antimicrobial drugs and/or cleaning of the housing area [31], because coronavirus is an opportunistic infectious agent.

Since more than one pathogen can be found in calf diarrhea, it is emphasized that the severity of the disease is higher in mixed infections [50]. The most common mixed infection was caused by the two agents viz: *Cryptosporidium* spp. and rotavirus (8%) in the present study. Previous studies also found that *Cryptosporidium* spp. and rotavirus were the most prevalent infectious agents in diarrheic calves [19, 33]. It has also been reported that *Cryptosporidium* spp., which is the main cause of neonatal calf diarrhea, is a risk factor for the emergence of rotavirus infection [40, 51]. In the etiology of diarrhea, various researchers have reported that coronavirus can play a role with or without rotavirus [35, 52]. Conrady *et al.* reported that the highest estimated mean *Cryptosporidium*–rotavirus prevalence was identified in Ireland (16.7%) [53], the highest estimated mean *Cryptosporidium*–coronavirus prevalence was detected in the United Kingdom (4.3%), and the highest estimated mean *Cryptosporidium*–*E. coli* prevalence in Turkey (4.7%). Alkan reported that seven out of 83 diarrheal calves (13.4%) had mixed infections with both rotavirus and coronavirus [35]. In a study of 82 diarrheic calves in Konya, three calves (3.6%) were tested positive for rotavirus and coronavirus [54]. In a different study involving acute diarrhea in 30 calves from 1–28 days of age, one calf was positive for rotavirus and *E. coli* at three weeks of age, and one for rotavirus and coronavirus at two weeks of age [52]. In research carried out by Icen *et al.*, the rates of various dual infections were 15.6% (*Cryptosporidium* spp. rotavirus), 1% (coronavirus– rotavirus), 5.2% (*E. coli* K99–*Cryptosporidium* spp.), and 7.3% (rotavirus–*E. coli*) [33]. Two different triple infection rates were reported as 3.1% (*Cryptosporidium* spp.–*E. coli* K99–rotavirus) and 1.0% (*Cryptosporidium* spp.– coronavirus–rotavirus). While *E. coli* and coronavirus infections were less common in this region, the frequency of rotavirus and *Cryptosporidium* spp. infections was found to be higher in calves less than 30 days old.

CONCLUSIONS

This study concludes that the etiologic agents alone or in combination may play a role in the frequency distribution of the neonatal calf diarrhea. Hence, treatment protocols should be designed with a consideration for mixed infections. The target agents are of primary zoonotic factors for human infections, with cattle assuming the role of reservoir host. Hence, the results of this study may be used in screening of infections in reservoir hosts and for the development of effective control strategies through better understanding of the transmission dynamics. Other tools like providing colostrum to the newborns and an awareness campaign can be useful as a preventive management of the neonatal calf diarrhea.

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Ethics approval

The study protocol was approved by Ethical Committee of Faculty of Veterinary Medicine, Selcuk University, Turkey (Approval no:14/03).

Conflict of interest

The authors declare that they have no conflict of interest.

BIBLIOGRAPHIC REFERENCES

[1] Lorenz I. Diarrhoea of the young calf: an update. Proceedings of the XXIV World Buiatrics Congress. Nice, Oct. 15, 2006, France. 2016; p 1–14.

[2] Millemann Y. Diagnosis of neonatal calf diarrhoea. Rev. Méd. Vét. 2009; 160(8/9):404–9. doi: https://doi.org/bspzp7

[3] Izzo M, Kirkland P, Mohler V, Perkins N, Gunn A, House J. Prevalence of major enteric pathogens in Australian dairy calves with diarrhoea. Aust. Vet. J. 2011; 89(5):167–73.

[4] İnci A. Sığırlarda Cryptosporidiosis, Ozcel MA. (ed). Veteriner Hekimliğinde Parazit Hastalıkları. 2013; 135–42.

[5] 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; 48:1–16. doi: https://doi.org/gbtg74

[6] Mickelsen WD, Evermann JF. *In utero* infections responsible for abortion, stillbirth, and birth of weak calves in beef cows. Vet. Clin. North Am. Food Anim. 1994; 10(1):1–14. doi: https://doi.org/kdrq

[7] Almeida PR, Lorenzetti, Cruz RS, Watanabe TT, Zlotowski P, Alfieri AA, Driemeier D. Diarrhea caused by rotavirus A, B, and C in suckling piglets from southern Brazil: molecular detection and histologic and immunohistochemical characterization. J. Vet. Diagn. Investig. 2018; 30(3):370–6. doi: https://doi.org/gdkms5

[8] Bok M, Alassia M, Frank F, Vega CG, Wigdorovitz A, Parreno V. Passive immunity to control Bovine coronavirus diarrhea in a dairy herd in Argentina. Rev. Argent. Microbiol. 2018; 50(1):23–30. doi: https://doi.org/jnmr

[9] Collery P. Causes of perinatal calf mortality in the Republic of Ireland. Ir. Vet. J. 1996; 49:491–6.

[10] Snodgrass D, Terzolo HR, Sherwood, Campbell I, Menzies J, Synge B. Aetiology of diarrhoea in young calves. Vet. Rec. 1986; 119(2):31–4. doi: https://doi.org/brz5st

[11] Ikemori Y, Ohta M, Umeda K, Icatlo Jr. FC, Kuroki M, Yokoyama H, Kodama Y. Passive protection of neonatal calves against bovine coronavirus–induced diarrhea by administration of egg yolk or colostrum antibody powder. Vet. Microbiol. 1997; 58(2–4):105–11. doi: https://doi.org/cwvzq6

[12] Blanco JE, Blanco M, Mora, Blanco J. Production of toxins (enterotoxins, verotoxins, and necrotoxins) and colicins by *Escherichia coli* strains isolated from septicemic and healthy chickens: relationship with *in vivo* pathogenicity. J. Clin. Microbiol. 1997; 35(11):2953–7. doi: https://doi.org/kdrr

[13] Butler DG, Clarke RC. Diarrhoea and dysentery in calves. In: Gyles CL (ed). *Escherichia coli* in domestic animals and humans. Wallingford (United Kingdom): CAB International Oxon. 1994; p. 91–116.

[14] Ok Ü, Girginkardeşler N, Kilimcioğlu A, Limoncu E. Dışkı inceleme yöntemleri. In: Özcel, MA, Altintas, N. Parazit Hastaliklarinda Tani. Türkiye Parazitoloji Derg. 1997; 15:1–61.

[15] Ekici Ö, Sevinç F, Coşkun A, Işık, Sevinç N. İshalli buzağılarda cryptosporidiosisin yaygınlığı. Eurasian J. Vet. Sci. 2011; 27(2):123–6.

[16] Nussbaum DJ, Salord JR, Rimmele DD. Evaluation of quantitative latex agglutination for detection of *Cryptosporidium parvum*, *E. coli* K99, and rotavirus in calf feces. J. Vet. Diagn. Invest. 1999; 11(4):314–8.

[17] Khan A, Khan MZ. Aetiopathology of neonatal calf mortality. J. Isl. Acad. Sci. 1991; 4 (2): 159–65.

[18] Ragsdale J. Diagnostic samples, tests for calf diarrhea. Vet. Quart. 2004; 7(1):6.

[19] Lanz UF, Kaufmann T, Sager H, Albini ., Zanoni R., Schelling E, Meylan M. Prevalence of four enteropathogens in the faeces of young diarrhoeic dairy calves in Switzerland. Vet. Rec. 2008; 163(12):362–6. doi: https://doi.org/cn739k

[20] Gillhuber J, Rügamer D, Pfister K, Scheuerle MC. Giardiosis and other enteropathogenic infections: a study on diarrhoeic calves in Southern Germany. B.M.C. Res. Notes. 2014; 7:1–9. doi: https://doi.org/f5xwbh

[21] Değerli S, Çeliksöz Kalkan K, Ozcelik S. Prevalence of *Cryptosporidium* spp. and *Giardia* spp. in cows and calves in Sivas. Turk. J. Vet. Anim. Sci. 2005; 29(4):995–9.

[22] Güven E Avcıoğlu H, Balkaya I, Hayirli A, Kar S, Karaer Z. Prevalence of Cryptosporidiosis and molecular characterization of *Cryptosporidium* spp. in calves in Erzurum. Kafkas Univ. Vet. Fak. Derg. 2013; 19(6):969–74. doi: https://doi.org/kdrs

[23] Gündüz N, Arslan MÖ. Kars Yöresinde Buzağılarda *Cryptosporidium* Enfeksiyonları Prevalansının Asit Fast Boyama (mAF) ve ELISA Yöntemleriyle Belirlenmesi. Turkiye Parazitol. Derg. 2017; 41:5–8. doi: https://doi.org/kdrt

[24] Yildirim A, Sevinc F, Onder Z, Duzlu O, Ekici OD, Isik N, Ciloglu A, Simsek , Yetismis G, Inci A. Comparison of three diagnostic methods in the diagnosis of cryptosporidiosis and *gp*60 subtyping of *Cryptosporidium parvum* in diarrheic calves in Central Anatolia Region of Turkey. EuroBiotech J. 2021; 5 2):63–9. doi: https://doi.org/kdrv

[25] Gow S, Waldner C. An examination of the prevalence of and risk factors for shedding of *Cryptosporidium* spp. and *Giardia* spp. in cows and calves from western Canadian cow–calf herds. Vet. Parasitol. 2006; 137(1–2):50–61. doi: https://doi.org/crfp9w

[26] Singh BB, Sharma R, Kumar H, Banga H, Aulakh RS, Gill JPS, Sharma JK, Prevalence of *Cryptosporidium parvum* infection in Punjab (India) and its association with diarrhea in neonatal dairy calves. Vet. Parasitol. 2006; 140(1–2):162–5. doi: https://doi.org/bt93g7

[27] Cai M, Guo Y, Pan B, Li N, Wang X, Tang C, Yaoyu F, Xiao L. Longitudinal monitoring of *Cryptosporidium* species in pre–weaned dairy calves on five farms in Shanghai, China. Vet. Parasitol. 2017; 241:14–9. doi: https://doi.org/gbkz2v

[28] Caffarena RD, Casaux ML, Schild CO, Fraga M, Castells M, Colina R, Maya L, Corbellini LG, Riet–Correa F, Giannitti F. Causes of neonatal calf diarrhea and mortality in pasture–based dairy herds in Uruguay: a farm–matched case–control study. Braz. J. Microbiol. 2021; 52(2):977–88. doi: https://doi.org/kdrw

[29] Hoque S, Mavrides DE, Pinto P, Costas S, Begum N, Azevedo–Ribeiro C, Liapi M, Kvac M, Malas S, Gentekaki E, Tsaousis AD. High occurrence of zoonotic subtypes of *Cryptosporidium parvum* in Cypriot dairy farms. Microorganisms. 2022; 10(3):531. doi: https://doi.org/kdrx

[30] Markovics A, Pipano E. Shedding of cryptosporidial oocysts by naturally infected calves. Isr. J. Vet. Med. 1987; 43:46–9.

[31] Bartels CJM, Holzhauer M, Jorritsma R, Swart WA, Lam TJ. Prevalence, prediction and risk factors of enteropathogens in normal and non–normal faeces of young Dutch dairy calves. Prev. Vet. Med. 2010; 93(2–3):162–9. doi: https://doi.org/ch7mpc

[32] Díaz–Lee A, Mercado R, Onuoha E., Ozaki L, Muñoz P, Muñoz V, Martinez FJ, Fredes F. *Cryptosporidium parvum* in diarrheic calves detected by microscopy and identified by immunochromatographic and molecular methods. Vet. Parasitol. 2011; 176(2–3):139–44. doi: https://doi.org/cvmxkh

[33] Içen H, Arserim NB, Işik N, Özkan C, Kaya A. Prevalence of Four Enteropathogens with Immunochromatographic Rapid Test in the Feces of Diarrheic Calves in East and Southeast of Turkey. Pak. Vet. J. 2013; 33(4): 496–99

[34] 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(42):1–6. doi: https://doi.org/gjf6zz

[35] Alkan F. Buzağı ishallerinde rotavirus ve coronavirusların rolü. Ankara. Üniv. Vet. Fak. Derg. 1998; 45(1):29–37. doi: https://doi.org/kdrz

[36] Sakli GU, Bulut O, Hasöksüz M, Hadimli HH. Investigation of bovine coronavirus and bovine rotavirus by rapid diagnosis kit and RT–PCR in diarrheic calf feces. J. Istanbul. Vet. Sci. 2019; 3(3):57–63. doi: https://doi.org/kdr2

[37] Atasoy MO, Isidan H, Turan T. Genetic diversity, frequency and concurrent infections of picobirnaviruses in diarrhoeic calves in Turkey. Trop. Anim. Health Prod. 2022; 54(2):127. doi: https://doi.org/kdr3

[38] Keleş İ, Ekinci G, Tüfekçi E, Citil M, Güneş V, Aslan Ö, Onmaz AC, Bekdik IK, Varol K, Deniz O. Etiological and predisposing factors in calves with neonatal diarrhea: A clinical study in 270 case series. Kafkas Univ. Vet. Fak. Derg. 2022; 28(3):315–326. doi: https://doi.org/kdr4

[39] De Graaf DC, Vanopdenbosch E, Ortega-Mora LM, Abbassi H, Peeters JE. A review of the importance of cryptosporidiosis in farm animals. Int. J. Parasitol. 1999; 29(8):1269–87. doi: https://doi.org/frgq9t

[40] Cruvinel LB, Ayres H, Zapa DMB, Nicaretta JE, Couto LFM, Heller LM, Bastos TSA, Cruz B, Soares VE, Teixeira WF, Oliveria JS, Fritzen JT, Alfieri AA, Freire R.L, Lopes WDZL. Prevalence and risk factors for agents causing diarrhea (Coronavirus, Rotavirus, *Cryptosporidium* spp., *Eimeria* spp., and nematodes helminthes) according to age in dairy calves from Brazil. Trop. Anim. Health Prod. 2020; 52:777–91. doi: https://doi.org/kdr5

[41] Mukhtar N, Yaqub T, Munir M, Nazir J, Aslam A., Masood A, Tahir Z, Javed M, Nadeem A. Prevalence of group a bovine rota virus in neonatal calves in Punjab, Pakistan. The J. Anim. Plant. Sci. 2017; 27(2):379–83.

[42] Küliğ C, Coşkun A. Sivas ve ilçelerindeki neonatal ishalli buzağılarda *E. coli*, *Cryptosporidium*, *Clostridium perfringens*, Rotavirüs ve Coronavirüs prevalansı. Turk. Vet. J. 2019; 1(2):69–73.

[43] Cengiz S, Adiguzel MC. Determination of virulence factors and antimicrobial resistance of *E. coli* isolated from calf diarrhea, part of eastern Turkey. Ankara Univ. Vet. Fak. Derg. 2020; 67 (4): 365–71. doi: https://doi.org/kdr6

[44] Garcıa A, Ruiz-Santa-Quiteria J, Orden J, Cid D, Sanz R, Gómez-Bautista M, Fuente R. Rotavirus and concurrent infections with other enteropathogens in neonatal diarrheic dairy calves in Spain. Comp. Immunol. Microbiol. Infect. Dis. 2000; 23(3):175–83. doi: https://doi.org/dp5mtg

[45] Ryu J, Kim S, Park J, Choi KS. Characterization of virulence genes in *Escherichia coli* strains isolated from pre-weaned calves in the Republic of Korea. Acta Vet. Scand. 2020; 62(1):1–7. doi: https://doi.org/h3mf

[46] Tsunemitsu H, Yonemichi H, Hirai T, Kudo T, Onoe S, Mori K, Shimizu M. Isolation of bovine coronavirus from feces and nasal swabs of calves with diarrhea. J. Vet. Med. Sci. 1991; 53(3):433–7. doi: https://doi.org/dm9nkk

[47] Gulliksen SM, Jor E, Lie K, Hamnes I, Løken T, Åkerstedt J, Østerås O. Enteropathogens and risk factors for diarrhea in Norwegian dairy calves. J. Dairy Sci. 2009; 92(10):5057–66. doi: https://doi.org/cffm8s

[48] Erdoğan HM, Ünver A, Güneş V, Çitil M. Frequency of rotavirus and coronavirus in neonatal calves in Kars district. Kafkas Univ. Vet. Fak. Derg. 2003; 9(1):65–8.

[49] Hasoksuz M, Kayar A, Dodurka T, IlgazA. Detection of respiratory and enteric shedding of bovine coronaviruses in cattle in Northwestern Turkey. Acta Vet. Hung. 2005; 53(1):137–46. doi: https://doi.org/dcp755

[50] Pospischil A. Pathologie und Pathogenese infektiöser Durchfallerkrankungen beim Kalb. Vet. 1989; 5:27–32.

[51] Garro CJ, Morici GE, Tomazic ML, Vilte D, Encinas M, Vega C, Bok M, Parreno V, Schnittger L. Occurrence of *Cryptosporidium* and other enteropathogens and their association with diarrhea in dairy calves of Buenos Aires Province, Argentina. Vet. Parasitol. Reg. Stud. Reports. 2021; 24:100567. doi: https://doi.org/kdr7

[52] Al M, Balıkçı E. Neonatal ishalli buzağılarda rotavirus, coronavirus, *E. coli* K99 ve *Cryptosporidium parvum'un* hızlı test kitleri ile teşhisi ve enteropatojen ile maternal immünite ilişkisi. F. Ü. Sağ. Bil. Vet. Derg. 2012; 26(2):73–8.

[53] Conrady B, Brunauer M, Roch FF. *Cryptosporidium* spp. Infections in Combination with Other Enteric Pathogens in the Global Calf Population. Anim. 2021; 11(6):1786. doi: https://doi.org/kdr8

[54] Ok M, Güler L, Turgut K, Ok Ü, Şen I, Gündüz I, Birdane MF, Guzelbektas H. The studies on the aetiology of diarrhoea in neonatal calves and determination of virulence gene markers of *Escherichia coli* strains by multiplex PCR. Zoon. Publ. Health. 2009; 56(2):94–101. doi: https://doi.org/fvg7v3