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Research Article

Prevalence and Molecular Characterization of *Cryptosporidium* in Dairy Cattle from Farms in Algeria

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Abstract

Faecal samples were collected from 338 less than 03 month-old dairy calves from 34 cattle herds located in three regions of north-eastern Algeria in order to characterize *Cryptosporidium* oocyst output using Ziehl-Neelsen, Heine and immunofluorescence methods and to identify different species of *Cryptosporidium* using a PCR 18S. Faecal samples were processed, firstly, through concentration and modified Ziehl-Neelsen staining techniques. Then, 93 faecal samples (not concentrated) were randomly selected and analyzed with the Heine staining technique. Positives samples revealed by both methods, Heine and Ziehl-Neelsen, were analyzed by direct immunofluorescent antibody test and by PCR 18S. Twenty-three herds out of thirty four (68%) were found positive and 108 (32%) calves were shedding oocysts. The prevalence of excretion peaked when calves were 15-30 days of age. However, it was found that the majority of infected calves were coupled to diarrhoea in 1-14 day old calves. Moreover, calves aged 1-14 days showed a higher level of oocyst shedding with OPG equal to 5.2×104. Three species were identified by sequencing of the 18S PCR products; *Cryptosporidium parvum, Cryptosporidium bovis* and *Cryptosporidium andersoni*. These results represent the first report on the genetic identification of *Cryptosporidium* species in dairy cattle in Algeria and confirm the frequent occurrence of *Cryptosporidium* in diarrhoeic calves.

Keywords: *Cryptosporidium, PCR 18S, calves, Algeria*

Cezayir Çiftliklerinde Sığırlardaki *Cryptosporidium* Prevalansı ve Moleküler Karakterizasyonu

Özet

Kuzey-doğu Cezayir'de 3 farklı bölgedeki 34 sığır çiftliğinden toplam 338 adet 3 aylıktan daha küçük buzağıya ait dışkı örnekleri Ziehl-Neelsen, Heine ve immunfloresan yöntemleri kullanılarak *Cryptosporidium* oositlerini karakterize etmek ve 18S PCR ile farklı türlerini belirlemek amacıyla toplandı. Dışkı örnekleri konsantrasyon ve sonrasında modifiye Ziehl-Neelsen yöntemi ile işlendi. Takibinde, 93 dışkı örneği (konsantre edilmemiş) kör olarak seçildi ve Heine boyama tekniği ile analiz edildi. Heine ve Ziehl-Neelsen tekniklerinin her ikisiyle birlikte pozitif bulunan örnekler direkt immunfloresan ve 18S PCR ile analiz edildi. Otuz dört çiftlikten 23'ü (%68) pozitif olarak belirlenirken 108 (%32) buzağının oosit yaydığı tespit edildi. Oosit yayma oranı buzağılar 15-30 günlükken en yüksek seviyedeydi. Ancak enfekte buzağıların çoğunun 1-14 günlükken ishalli oldukları belirlendi. 1-14 günlük buzağılar her bir gram dışkı örneğinde 5.2×104 oosit ile daha yüksek seviyede oosit yayma oranı gösterdiler. 18S PCR ile *Cryptosporidium parvum, Cryptosporidium bovis* ve *Cryptosporidium andersoni* olmak üzere üç tür belirlendi. Bu çalışma ile Cezayir'de sütçü sığırlarda *Cryptosporidium* türlerinin ilk defa genetik identifikasyonu yapılarak ishalli buzağılardaki bulunma sıklığı tespit edildi.

Anahtar sözcükler: *Cryptosporidium, PCR 18S, buzağı, Cezayir*

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INTRODUCTION

Cryptosporidium is a major agent of neonatal diarrhoea of calves and infection has been associated with economic losses due to the occurrence of the diarrhoea and more rarely to the death of animals [1].

Cryptosporidium was first isolated in cattle in 1971 [2]. Since then, there have been numerous reports of cryptosporidiosis in different countries [3-6]. More than 20 *Cryptosporidium* species have been described. Cattle have been reported to be infected with four species of *Cryptosporidium*: *Cryptosporidium parvum, Cryptosporidium bovis, Cryptosporidium ryanae* and *Cryptosporidium andersoni* [3,7-9].

To date, little information regarding the epidemiology of *Cryptosporidium* infection in cattle from Algeria has been reported and no studies have been conducted to identify the different *Cryptosporidium* species. The studies realized by Akam et al.[10], Khelef et al.[11] and Ouchene et al.[12] in Algeria, indicate only a prevalence of *Cryptosporidium* infection in cattle (from 16.66% to 24.7%) using Ziehl-Neelsen acid-fast method but no species have been identified.

Consequently, the present study was designed to assess the prevalence of *Cryptosporidium* in dairy calves and, for the first time in Algeria, as a preliminary approach, to try to identify different species of *Cryptosporidium* using PCR assay.

MATERIAL and METHODS

Samples

This study was performed in north Algeria during the period between January and December 2010. Samples were taken from thirty-four dairy cattle herds: 12 from Setif region, 15 from El Tarf region and 7 from Constantine region. Faecal samples were collected once from a total of 338 calves aged less than 3 month-old of which 105, 165 and 68 were sampled in Setif, El Tarf and Constantine regions, successively.

Faecal samples were taken directly from rectum and transported directly to the laboratory in a cool box to be stored and processed in the following 3 days.

Laboratory Analyses

- Oocyst concentration: Faecal samples (n=388) were concentrated as previously described by Castro-Hermida et al.[13]. The sediment was removed and washed in distilled water by centrifuging at 1000× g for 5 min at 4°C. The volume of the oocyst suspension was adjusted to 1 ml using distilled water.

- Ziehl-Neelsen acid-fast method: The oocyst suspension was used to prepare thin faecal smear on slide for staining with Ziehl-Neelsen technique modified by Henriksen and Pohlenz [14]. After staining, faecal smears were observed under an optical microscope at 1000×.

The intensity of excretion was evaluated semi-quantitatively according to the average number of oocysts in 30 randomly selected fields: 0: absence of oocyst; +1: <1oocysts per field; +2: 1-10 oocysts per field; +3: >10 oocysts per field.

- Heine staining method: From the 338 faecal samples,93 (not concentrated) were randomly selected to be analyzed with the Heine staining technique [15]. The intensity of excretion was also evaluated semi-quantitatively according to the average number of oocysts in 10 randomly selected fields: 0: absence of oocyst, 1: <1 oocyst per field, 2: 1-10 oocysts per field, 3: 11-20 oocysts per field, 4: 21-30 oocysts per field, 5: >30 oocysts per field.

- Immunofluorescence examination and molecular characterization: Positives samples revealed by both methods Heine and Ziehl-Neelsen (n=16), were analyzed by direct immunofluorescent antibody test (IFAT) (MeriFluor® *Cryptosporidium/Giardia*, Meridian Bioscience Europe, Nice, France) to calculate the number of oocysts per gram of faeces (OPG) and by PCR 18S to identify different species of *Cryptosporidium*.

For the IFAT, 10 µl of oocyst suspension was fixed on the slides using acetone at 4°C for 10 min. The slides were then processed according to the manufacturer's instructions. The oocysts were observed 400 × magnifications under fluorescence microscopy. The OPG was calculated by using this formula: number of oocysts seen in the well × 100/2.

For the PCR 18S, 250 µl of the oocyst suspension was processed using the UltraClean Faecal DNA Isolation Kit (MO BIO Laboratories, Inc, Carlsbad, USA) according to the manufacturer's instructions. The eluted DNA was dissolved in 50 µl.

A two-step nested PCR protocol was used to amplify an 830 bp segment of the 18S rRNA gene using primers 5'-TTCTAGAGCTAATACATGCG-3' and 5'-CCCATTTCCTTCGAA ACAGGA-3' for primary PCR and 5'-GGAAGGGTTGTATTTAT TAGATAAAG-3' and 5'-AAGGAGTAAGGAACAACCTCCA-3' for secondary PCR [16,17].

The amplification runs were performed in a Bio-Rad iCycler thermal cycler as: 10 min at 94°C, 40 cycles of 45 s at 94°C, 55 s at 55°C and 1 min at 72°C, with a final 5min elongation step at 72°C. Amplification products (10 µl) were analyzed on 2% agarose gel and visualized after ethidium bromide staining under UV light.

PCR products were sequenced using an ABI 3730XL sequencer (Applied Biosystems, Warrington, UK). Sequence accuracy was ensured by two-directional sequencing, and all electrophoregrams were manually checked and edited as deemed necessary. Sequences were compared with

known sequences by BLAST-analysis against the NCBI database.

Statistical Analysis

The Chi-square test was used to compare between the different prevalences and Pearson correlation test was used to compare between the results of both methods Ziehl-Neelsen and Heine. The statistical program used was R i386 3.0.2 for Windows GUI front-end. Differences were considered as significant when p value was less than 0.05.

RESULTS

Ziehl-Neelsen Acid-fast Method

Twenty-three herds out of thirty-four (68%) were found positive and 108 (32%) calves were shedding *Cryptosporidium* oocysts. Within-herd prevalence of infection ranged from 6% to 100% and in different regions, varies from 28% (29/105), 30% (49/165) and 44% (30/68) in Setif, El Tarf and Constantine, respectively.

Prevalence of infection showed the highest values of 46% in the 15-30 day-old calf group when compared to other age groups (P<0.001) *(Table 1)*.

The overall percentage of diarrhoeic calves at the time of sampling was 20% (67/338). This general figure varied according to the age and showed a higher value (28%) for the 1-14 day-old calves compared to the other age groups (P<0.01). The majority of infected calves were coupled to diarrhoea in 1-14 day old calves (59%) compared with other age groups (P<0.001) *(Table 1)*.

A significantly higher level of oocysts shedding (3+) was observed in 1-14 day-old animals compared to the other levels (+1 and +2) (P<0.01). In contrast, in the other age groups (15-30 days and 31-90 days), the oocysts excretion level was low (+1) (P<0.01). The same observations have been reported in diarrhoeic calves *(Table 1)*.

Heine Staining Method

Heine staining method showed that 19% (18/93) of calves were infected; this prevalence is lower than that reported by Ziehl-Neelsen method above (P<0.001).

However, a correlation was observed between the prevalence of *Cryptosporidium* infection reported by Ziehl-Neelsen method and that reported by Heine method according to age of calves (P<0.01).

Moreover, *Cryptosporidium* was commonly found among 15-30-day-old calves (35%) (P<0.05) and1-14day-old calves were found to be most affected by diarrhoea (55%) with a prevalence of *Cryptosporidium* infection of 100% in diarrhoeic calves (P<0.01). The intensity of oocyst shedding (2-5) was the highest in 1-14 day-old calves compared to other age groups *(Table 2)*.

Immunofluorescence Examination

The mean oocyst excretion calculated using the IFAT indicated that calves 1-14 days-old were the most excreting of *Cryptosporidium* oocysts with OPG equal to 5.2×10^4. This result was in accordance with the finding of Ziehl-Neelsen and Heine methods. The OPG in 15-30 days and 31-90 day-old calves was 2.4×10^4 and1.6×10^2, respectively.

Molecular Characterization

Three species were identified by sequencing of the 18S PCR products; *Cryptosporidium parvum* in only one 20-day old calf, *Cryptosporidium bovis* in two calves 21 and 60 days

Table 1. *Prevalence and intensity of excretion of Cryptosporidium oocysts according to age of calves and to the score of diarrhoea (Ziehl-Neelsen methods)*
Tablo 1. *Yaşa ve ishal derecesine göre Cryptosporidium oositlerinin atılma prevalansı ve sıklığı (Ziehl-Neelsen metodu)*

Age Groups	Overall Diarrhoea Frequency	Cryptosporidiosis Individual Prevalence	% of Diarrhoeic Positive Calves	Oocyst excretion level				Oocyst Excretion Level from Diarrhoeic Calves			
				0	+1	+2	+3	0	+1	+2	+3
1-14 d (n=72)	28% (20/72)	37% (27/72)	59% (16/27)	62% (45/72)	19% (5/27)	30% (8/27)	52% (14/27)	9% (4/45)	6% (1/16)	31% (5/16)	62% (10/16)
15-30 d (n=83)	23% (19/83)	46% (38/83)	39% (15/38)	54% (45/83)	47% (18/38)	29% (11/38)	24% (9/38)	9% (4/45)	40% (6/15)	33% (5/15)	27% (4/15)
31-90 d (n=183)	15% (28/183)	23% (43/183)	26% (11/43)	76% (140/183)	70% (30/43)	16% (7/43)	14% (6/43)	12% (17/140)	55% (6/11)	36% (4/11)	9% (1/11)

Table 2. *Prevalence, intensity of excretion of Cryptosporidium oocysts according to the age of calves (Heine method)*
Tablo 2. *Yaşa göre Cryptosporidium oositlerinin atılma prevalansı ve sıklığı (Heine metodu)*

Age Categories	Overall Diarrhoea Frequency	Prevalence of Excretion	Proportions of Diarrhoeic Positive Calves	Intensity of Excretion
1-14 days (n=22)	55% (12/22)	18% (4/22)	100% (4/4)	2-5
15-30 days (n=31)	39% (12/31)	35% (11/31)	73% (8/11)	1-3
31-90 days (n=40)	45% (18/40)	7% (3/40)	0% (0/3)	1-2

old and *Cryptosporidium andersoni* in only one 35 day old calf.

DISCUSSION

Cryptosporidium parvum is one of the major causes of neonatal calf diarrhea [1,12,18]. However, so far, studies on the prevalence of *Cryptosporidium* in Algeria [10,12] have been conducted without molecular characterization.

The present study indicates that *Cryptosporidium* remains widespread in Algeria. The results of Ziehl-Neelsen method showed that 68% (23/34) of dairy cattle were infected and the prevalence of *Cryptosporidium* infection in calves was 32% (28%-44%) which is similar to the results reported by other authors in Algeria [10-12] and Spain [18] and higher to the results showed in Turkey [19-21].

However, our results showed that the parasite prevalence on farms ranged from 6.25% to 100% of the sampled animals. This observation was in accordance with results in France [22] and Michigan (USA) [23].

In order to increase the sensitivity of the assays, the concentration method was utilized [12,24] which explains, in the present study, the great prevalence revealed by Ziehl-Neelsen technique (32%) compared to that reported by Heine technique (19%). However, according to the age calves, a correlation of the prevalence of *Cryptosporidium* infection was observed between the two techniques ($P<0.01$).

Higher frequency of oocyst shedding was observed in 15-30 day-old calf groups (both Ziehl-Neelsen and Heine methods) what is a steady and characteristic trait of *Cryptosporidium* infection in young cattle [25]. The same results were reported in Algeria [10,12]. Decreasing prevalence of *Cryptosporidium* infection with age in cattle has been constantly observed [19,26,27].

Our results showed that the prevalence of *Cryptosporidium* infection in 1-14 day-old calves was significantly associated with diarrhoea and high level of oocysts shedding. This suggested a highly significant association between the intensity of shedding and the presence of diarrhea in young calves. Such positive relationship between diarrhoea and *Cryptosporidium* oocyst output has been described in calves with simultaneous occurrence of diarrhoea and high oocyst counts [26].

The peak of cryptosporidiosis prevalence in young calves could reflect the immaturity of the immune status and the low excretion of *C. parvum* oocysts in older calves might be related to the development of immunity that also protected the animal against a secondary infection [28].

Cattle have been reported to be infected with four species of *Cryptosporidium*: *Cryptosporidium parvum*, *Cryptosporidium bovis, Cryptosporidium ryanae* and *Cryptosporidium andersoni* [9]. Our study represents the first report on the genetic identification of *Cryptosporidium* species in dairy cattle in Algeria. Three species were identified; *Cryptosporidium parvum*, *Cryptosporidium bovis* and *Cryptosporidium andersoni*.

Several authors reported that the species *C. parvum* constituted the majority of infections in pre-weaned calves whereas *C. bovis* in post-weaned calves and *C. andersoni* in adult cows [7,26,29-32]. In our study, *C. parvum* was identified in only one 20 day-old calf, *C. bovis* in two calves 21 and 60 day-old and *C. andersoni* in only one 35 day old calf.

In Belgium, *C. parvum* is predominant in young dairy calves (<1month) [33]. *C. bovis* was found in dairy calves at 2 or 3 weeks of life in India and China [27].

Our study confirms the frequent occurrence of *Cryptosporidium* in diarrhoeic calves in Algeria. As several species and/or genotypes may be involved according to the different age classes of cattle, further studies are needed to have a better understanding of *Cryptosporidium* infection in cattle farms and its zoonotic potential. Hence, considerable attention should be paid to preventing the spread of the infection.

These results represent the first report on the prevalence and genetic identification of *Cryptosporidium* species in dairy cattle in Algeria and may contribute to a better understanding of *Cryptosporidium* epidemiology in cattle.

However, calf neonatal diarrhoea in the field is a more complex and multifactorial disease that involves *Cryptosporidium* and other pathogens exposure as well as environmental and management factors and this should be considered.

REFERENCES

1. Cacciò SM, Pozio E: Advances in the epidemiology, diagnosis and treatment of cryptosporidiosis. *Expert Rev Anti-Infect Ther,* 4, 429-443, 2006. DOI: 10.1586/14787210.4.3.429

2. Panciera RJ, Thomassen RW,Garner FM: Cryptosporidial infection in a calf. *Vet Pathol*, 8, 479-484, 1971.

3. Halim NA, Plutzer J, Bakheit MA, Karanis P: First report of *Cryptosporidium* deer-like genotype in Malaysian cattle. *Vet Parasitol,* 152, 325-329, 2008. DOI: 10.1016/j.vetpar.2007.12.035

4. 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,* 137, 50-61, 2006 DOI: 10.1016/J.VETPAR.2005.05.071

5. Castro-Hermida JA, Almeida A, González-Warleta M, Correia da Costa JM, Rumbo-Lorenzo C, Mezo M: Occurrence of *Cryptosporidium parvum* and *Giardia duodenalis* in healthy adult domestic ruminants. *Parasitol Res,* 101, 1443-1448, 2007. DOI: 10.1007/s00436-007-0624-6

6. Nuchjangreed C, Boonrod K, Ongerth J, Karanis P: Prevalence and molecular characterization of human and *bovine Cryptosporidium* isolates in Thailand. *Parasitol Res*, 103, 1347-1353, 2008. DOI: 10.1007/s00436-008-1139-5

7. Fayer R, Santín M, Trout JM, Greiner E: Prevalence of species and

genotypes of *Cryptosporidium* found in 1-2-year-old dairy cattle in the eastern United States. *Vet Parasitol*, 135, 105-112, 2006. DOI: 10.1016/j.vetpar.2005.08.003

8. Fayer R, Santin M, Trout JM: Prevalence of *Cryptosporidium* species and genotypes in mature dairy cattle on farms in Eastern United States compared with younger cattle from the same locations. *Vet Parasitol*, 145, 260-266, 2007. DOI: 10.1016/j.vetpar.2006.12.009

9. Wyatt CR, Riggs MW, Fayer R: Cryptosporidiosis in neonatal calves. *Vet Clin Food Anim,* 26, 89-103, 2010. DOI: 10.1016/j.cvfa.2009.10.001

10. Akam A, Khelef D, Kaidi R, Abdulhussain Maria S, Şuteu E, Cozma V: Epidémiologie de la Cryptosporidiose bovine dans une région de Mitidja de l'Algérie. *Sci Parasitol*, 2, 22-27, 2002

11. Khelef D, Saib MZ, Akam A, Kaidi R, Chirila V, Cozma V, Adjou KT : Epidémiologie de la cryptosporidiose chez les bovins en Algérie. *Rev Méd Vét*, 158, 260-264, 2007.

12. Ouchene N, Ouchene-Khelifi NA, Aissi M, Benakhla A: Prévalence de *Cryptosporidium* spp. et *Giardia* spp. chez les bovins de la région de Sétif au nord-est de l'Algérie. *Rev elev med vet pays trop,* 65 (3-4): 53-56, 2012.

13. Castro-Hermida JA, Pors I, Poupin B, Ares-Mazás E, Chartier C: Prevalence of *Giardia duodenalis* and *Cryptosporidium parvum* infections in goat kids in western France. *Small Rumin Res*, 56, 259-264, 2005. DOI: 10.1016/j.smallrumres.2004.06.007

14. Henriksen SA, Pohlenz JFL: Staining of cryptosporidia by a modified Ziehl-Neelson technique. *Acta Vet Scand*, 22, 594-596, 1981.

15. Heine J: Eine einfache Nachweismethod fur Kryptosporidien in Kot. *Zentralbl Veterinaermed Reihe B,* 29, 324-327, 1982. DOI: 10.1111/j.1439-0450.1982.tb01233.x

16. 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,* 65, 3386-3391, 1999.

17. Xiao L, Bern C, Limor J, Sulaiman I, Roberts J, Checkley W, Cabrera L,Gilman RH, Lal AA: Identification of 5 types of *Cryptosporidium* parasites in children in Lima, Peru. *J Infect Dis,* 183, 492-497, 2001. DOI: 10.1086/318090

18. Quilez J, Sanchez-Acedo C, Del Cacho E, Clavel A, Causape AC: Prevalence of *Cryptosporidium* and *Giardia* infections in cattle in Aragon (Northeastern Spain). *Vet Parasitol,* 66, 139-146, 1996. DOI: 10.1016/s0304-4017(96)01015-1

19. Arslan MÖ, Gıcık Y, Erdoğan HM, Sarı B: Prevalence of *Cryptosporidium* spp. oocysts in diarrhoeic calves in Kars Province, Turkey. *Turk J Vet Anim Sci*, 25, 161-164, 2001.

20. Sarı B, Aktaş MS, Arslan MÖ: The prevalence of *Cryptosporidium* spp. in calves in Erzurum province. *Türkiye Parazitol Derg*, 32, 116-119, 2008. DOI: 10.9775/kvfd.2007.38-A

21. Arslan MO, Sarı B, Kara M, Taşçı GT, İtik Ekinci A, Gündüz N: Research on the prevalence of *Eimeria* and *Cryptosporidium* species in cows in periparturient period in Kars region. *Kafkas Univ Vet Fak Derg,* 18 (Suppl-A): A65-A70, 2012. DOI: 10.9775/kvfd.2011.5993

22. Follet J, Guyot K, Leruste H, Follet-Dumoulin A, Hammouma-Ghelboun O, Certad G, Dei-Cas E, Halama P: *Cryptosporidium* infection in a veal calf cohort in France: Molecular characterization of species in a longitudinal study. *Vet Res,* **42**, 116, 2011. DOI: 10.1186/1297-9716-42-116

23. Peng MM, Wilson ML, Holland RE, Meshnick SR, Lal AA, Xiao L: Genetic diversity of Cryptosporidium spp. in cattle in Michigan: Implications for understanding the transmission dynamics. *Parasitol Res,* **90,** 175-180, 2003**.** DOI: 10.1007/s00436-003-0834-5

24. Allen AVH, Ridley DS: Further observations on the formol ether concentration technique for faecal parasites. *J Clin Pathol,* 23, 545-546, 1970.

25. Castro-Hermida J, González-Losada Y, Mezo-Menéndez M, Ares-Mazás E: A study of cryptosporidiosis in a cohort of neonatal calves. *Vet Parasitol,* 106, 11-17, 2002. DOI: 10.1016/S0304-4017(02)00038-9

26. Santin M, Trout JM, Xiao L, Zhou L, Greiner E, Fayer R: Prevalence and age-related variation of *Cryptosporidium* species and genotypes in dairy calves. *Vet Parasitol,* 122, 103-117, 2004. DOI: 10.1016/j.vetpar.2004.03.020

27. Feng Y, Ortega Y, He G, DasP, Xu M, Zhang X, Fayer R, Gatei W, Cama V, Xiao L: Wide geographic distribution of *Cryptosporidium parvum* and deer like genotype in bovines. *Vet Parasitol*, 144, 1-9, 2007. DOI: 10.1016/J.VETPAR.2006.10.001

28. Fayer R, Gasbarre L, Pasquali P, Canals A, Almeria S, Zarlenga D: *Cryptosporidium parvum* infection in bovine neonates: Dynamic clinical, parasitic and immunologic patterns. *Int J Parasitol,* 28, 49-56, 1998. DOI: 10.1016/S0020-7519(97)00170-7

29. Santín M, Trout JM, Fayer R: A longitudinal study of cryptosporidiosis in dairy cattle from birth to 2 years of age. *Vet Parasitol*, 155, 15-23, 2008. DOI: 10.1016/j.vetpar.2008.04.018

30. Arslan MO, İtik Ekinci A: Determination of *Cryptosporidium parvum* subtypes in cattle in Kars province of Turkey. *Kafkas Univ Vet Fak Derg,* 18 (Suppl-A): A221-A226, 2012. DOI: 10.9775/kvfd.2012.6565

31. Tanrıverdi S, Arslan MÖ, Akiyoshi DE, Tzipori S, Widmer G: Identification of genotypically mixed *Cryptosporidium parvum* populations in humans and calves. *Mol Biochem Parasitol*, 130, 13-22, 2003. DOI: 10.1016/S0166-6851(03)00138-5

32. Tanrıverdi S, Markovics A, Arslan MÖ, İtik A, Shkap V, Widmer G: Emergence of distinct genotypes of *Cryptosporidium parvum* in structured host populations. *Appl Enviro Microbiol,* 72, 2507-2513, 2006. DOI: 10.1128/AEM.72.4.2507-2513.2006

33. Geurden T, Thomas P, Casaert S, Vercruysse J, Claerebout E: Prevalence and molecular characterisation of *Cryptosporidium* and *Giardia* in lambs and goat kids in Belgium. *Vet Parasitol,* 155, 142-145, 2008. DOI: 10.1016/j.vetpar.2008.05.002