Cryptosporidium parvum and Cryptosporidium andersoni infection in naturally infected cattle of northwest Iran

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Abstract

The protozoan intestinal parasite Cryptosporidium commonly infects cattle throughout the world and Iran. The present study was undertaken to determine the abundance and associated risk factors of Cryptosporidium infection in cattle herds of northwestern Iran. A total number of 246 fecal samples from 138 (56.1%) diarrheic (D) and 108 (43.9%) non-diarrheic (ND) cattle were randomly collected and examined by fecal smears stained with Ziehl-Neelsen. For molecular specification, DNA was extracted from collected Cryptosporidium oocysts and a fragment of 1325 bp in size from 18S RNA gene was amplified. The overall prevalence of Cryptosporidium infection was 22.3% (55/246). The prevalence of Cryptosporidium infection in examined calves less than 6 month-old was significantly higher than adult cattle. C. parvum and C. andersoni were identified in 20.3% (50/246) and 2.03% (5/246) of examined cattle, respectively. The highest prevalence of C. parvum infection was found in D calves <6 month-old (13.4%, 33/246), while C. andersoni was only detected in ND cattle (8.9%, 22/246). There was no significant difference in the prevalence between male than female cattle. There was no significant difference between prevalence and seasons of investigation. It was concluded that C. parvum was the prevalent species in younger animals compared to older ones as a potentially zoonotic agent in the region.

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Introduction

Cryptosporidium is an obligate intracellular protozoan parasite is a frequent cause of intestinal, gastric or respiratory cryptosporidiosis in a wide range of animals and humans hosts worldwide. Some of the zoonotic Cryptosporidium species usually causes self-limiting diarrhea in humans and animals. Cryptosporidiosis was reported for the first in 1971, as well as from Iranian cattle in 1984, and increasingly in a range of hosts. Over the past two decades, cattle have been identified as a common reservoir host for Cryptosporidium species. Currently, 20 different species of Cryptosporidium has been reported which C. parvum, C. bovis, C. ryanae, Cryptosporidium deer-like genotype, and C. andersoni are considered as cattle adapted. Of those, the intestinal specie C. parvum has zoonotic potential and is a frequent cause of human cryptosporidiosis. In terms of economic losses, C. parvum is considered as the most important species among cryptosporidial agents. Cryptosporidium andersoni as an abomasal parasite has been associated with reduced milk yield in dairy cattle and decreased weight gain in post weaned calves.

According to Xiao et al., a high diversity of Cryptosporidium genus based on several molecular markers, the 18S rRNA gene, has been shown by multilocus DNA analysis. In Iran, molecular detection of Cryptosporidium in humans has been undertaken by several researchers. While, to date, the molecular study for confirming cattle Cryptosporidium infection has not been investigated in this part of Iran. Additionally, the nationwide epidemiological survey is essential for the exact knowledge of infection status of C. parvum and C. andersoni in the country. Therefore, it is important to determine bovine Cryptosporidium infection and major risk factors in order to screen C. parvum and C. andersoni harboring in cattle herds of northwestern Iran.

Materials and Methods

Study area. The study area (West Azarbaijan province, WAP) is located in northwest of Iran with two rainy seasons, the first from March to May and the second in October–November. The study was carried out in the mountainous, mountainside and plain areas of Urmia suburb covering 355 villages that fall within a radius of 20 to 125 km of the city center (Fig. 1). According to the Iranian Veterinary Organization, an average population of eight million cattle is distributed in Iran; the WAP has approximately 6.1% of these cattle.

Animals. During the course of the study (September 2010 to August 2011), a total number of 101 cattle and 145 calves from 16 herds (375 cattle) were randomly selected from cattle herds in Urmia suburban of WAP. The herds examined were raised following traditional husbandry practices, with animals being mainly cross-bred and indigenous crossbred. The major risk factors for Cryptosporidium infection were host, Cryptosporidium species, and environmental factors (Table 1). The cattle were divided into four age groups, numbered and subjected to clinical examination. The age was estimated on the basis eruption of permanent incisor teeth. The consistency (Fc) of fecal specimens (D or ND) were recorded. The sample size for determining prevalence was estimated based on the formula (expected prevalence 30%, level of confidence 95%, and precision 5%) presented by Thrusfield.

Sample processing and examination. In each farm and herd, an amount of 25 g fresh fecal samples was collected directly from the rectum of each individual cattle and or calves and fecal smears were prepared. The Cryptosporidium oocysts were initially screened using the modified Ziehl-Neelsen staining method. The parasite species was identified by morphologic criteria which measured at 1000× magnification. Morphologically, C. parvum (5.0 × 4.5 µm) oocysts were discriminated from C. andersoni oocysts (7.6 × 5.6 µm).

Molecular procedures. Since, the modified Ziehl-Neelsen staining is a non-specific staining procedure the PCR procedure was performed to specify genus Cryptosporidium. For this purpose, oocysts were purified from fecal specimens of infected cattle using sucrose gradients as described by Arrowood and Sterling and subjected to molecular analysis. To rupture Cryptosporidium oocysts, 10 times freeze-thaw cycles were performed using liquid nitrogen. Genomic DNA was extracted by modified phenol-chloroform method using cetyltrimethylammonium bromide (CTAB) at 60 °C for 1 hr.
A fragment of 1325 bp of the 18S rRNA gene of Cryptosporidium was amplified using two primers (Crypto-sense: 5' TTCTAGAGCTAATACATGCG-3' and Crypto-antisense: 5' CCCTATTCCTCTGAAACAGGA -3'). \(^{23}\) PCR reaction was carried out in a 25 µL reaction mixture containing 3 µL of genomic DNA (diluted 1:30), 0.5 µL of Taq DNA polymerase (Fermentas, Munich, Germany), 4 µL of 1.25 mM dNTPs (CinnaGen, Tehran, Iran), 1 µL of 50 mM MgCl\(_2\), 2.5 µL of 10X PCR reaction buffer, 1 µL of each primer (25 µM). The samples were subjected to an initial denaturation step at 94 °C for 5 min, followed by 35 cycles of 45 sec at 94 °C, 45 sec at 55 °C, and 90 sec at 72 °C, and a final extension step at 72 °C for 7 min. The PCR product was analyzed by electrophoresis on 1.5% (w/v) agarose gel and visualized by staining with 1% ethidium bromide.

**Statistical analysis.** Statistical evaluation was undertaken to compare obtained data with confidence interval of 95% using non-parametric Fisher's exact test (Version 14.0; SPSS Inc., Chicago, IL, USA). Probability values of \( p < 0.05 \) were regarded statistically significant.

**Results**

The detected Cryptosporidium oocysts were nearly spherical in shape and contained four sporozoites. Based on the size, C. andersoni oocysts were morphologically distinguishable from C. parvum oocysts. The average diameter for C. parvum oocysts ranged from 4.5 to 5.0 µm and shape index (SI) as 1.1 ± 0.2, whereas larger C. andersoni oocysts ranged from 6.5 to 7.9 µm in size with SI as 1.3 ± 0.4.

The prevalence of C. parvum and C. andersoni infection in D or ND cattle and or calves of different age groups have been shown in Table 2. The overall prevalence of Cryptosporidium infection was 22.3% (55/246) which confirmed by PCR (Fig. 2). Out of them, 20.3% calves (50/246) and 20% cattle (5/246) were infected with C. parvum and C. andersoni, respectively (Table 2). The prevalence of Cryptosporidium infection in calves was significantly higher than that of adult cattle (\( p = 0.025 \)). The prevalence of Cryptosporidium infection was also significantly higher in 13.4% (33/246) D than 8.9% (22/246) ND cattle (\( p = 0.025 \)). Among the samples that were positive for Cryptosporidium species, 29 out of 246 (11.7%) were from male and 26 out of 246 (10.5%) were from female cattle (\( p = 0.026 \)). The highest prevalence of C. parvum infection was found in D calves (13.4%, 33/246), while C. andersoni was only detected in ND cattle (8.9%, 22/246) examined in which grazed in plain areas of the region (\( p = 0.0001 \)). Cryptosporidium parvum were detected in all examined herds while C. andersoni only detected in three herds (18.8%). Mixed infection with both Cryptosporidium species was also found in 2.4% (6/246) of infected cattle. There was no significant difference between the prevalence and seasons of investigation (\( p > 0.05 \)).

**Discussion**

Based on morphological characterization described by other researchers,\(^ {24,25} \) it was revealed that cattle in the region harbored at least two Cryptosporidium species. The small oocysts (mean size: 4.3 ± 0.2 µm) were identified as C. parvum (20.3%) and the large ones (average size: 6.8 ± 0.3 µm) were as C. andersoni (2.0%). Cryptosporidium parvum has been reported to be prevalent in neonates worldwide\(^ {14,26} \) and was not found in older cattle in any examined herds of the region. Therefore, the higher infection rate of C. parvum in current study compared to that of C. andersoni appears to reflect the dominance of C. parvum. Cryptosporidium andersoni has been reported for the first time in Iran by Sohrabi Haghdust.\(^ {8} \) Thus, this was the first report of C. andersoni occurrence in cattle of northwestern Iran. In this work, no C. andersoni oocysts were detected in cattle < 1 years old, supporting other reports that chronic C. andersoni infection usually occurs in adult cattle with no clinical symptoms.\(^ {14,21,27} \) The prevalence of the abomasal species C. andersoni was reported to be high in adult cattle while it is less pathogenic.\(^ {26-29} \)

Cryptosporidium distribution pattern and prevalence have been reported in many countries throughout the world\(^ {25,30} \) and Iran.\(^ {31,32} \) The results of the present work revealed that the estimated prevalence was similar to those reported in the previous researches.\(^ {14,33} \) Reported Cryptosporidium infection prevalence by other researchers varied from 22.0 to 59.0% worldwide\(^ {25} \) and 3.8 to 42.8% in Iran.\(^ {7,14,31,32} \) Seasonal changes in prevalence of Cryptosporidium infection were observed in spring and summer in the region. However, there was no significant association. In India, the highest prevalence of Cryptosporidium infection in cattle was reported in rainy season followed by summer
Table 1. Geographical distribution and major risk factors of cattle sampled from Urmia suburban of WAP, Iran.

| Study area (No. of cattle) | Season | No. of herds | No. of examined cattle | Fecal consistency | Age (year) | Sex | Geographical feature |
|----------------------------|--------|--------------|------------------------|-------------------|------------|-----|---------------------|
|                            |        |              |                        | <6*               | >6 - 3   | 4 - 6 | >6 | <6 | >6 | 4 - 6 | >6 | M | F | Mo | Ms | P |
| Nushan (12)                | Fall   | 1            | 4                      | 2 0 0 0 0 1 0 1 2 | 0 1 1 1 3 | + - - |
| Daresanji (8)              |        |              | 3                      | 1 0 1 0 0 1 1 0 2 | 1 1 - + - |
| Band (16)                  |        |              | 5                      | 1 0 2 0 1 0 0 1 2 | 1 1 2 - + - |
| Nushan (19)                | Winter | 1            | 8                      | 4 2 1 0 0 0 0 6 1 | 1 0 4 4 - - |
| Daresanji (21)             |        |              | 8                      | 4 2 0 1 0 0 0 6 1 | 1 0 3 5 - - |
| Band (23)                  |        |              | 15                     | 7 4 2 1 0 1 0 1 1 | 1 1 2 4 - - |
| Ghasebani (20)             | Spring | 1            | 10                     | 4 2 0 0 3 0 0 1 2 | 1 0 5 5 - - |
| Bardasur (29)              |        |              | 23                     | 11 4 3 0 2 0 3 1 5 | 3 2 3 4 - - |
| Gharaghaj (33)             |        |              | 26                     | 16 1 0 3 3 3 0 6 1 | 2 3 2 4 - - |
| Imamkandi (27)             |        |              | 18                     | 10 0 1 0 1 0 0 6 1 | 0 1 2 0 - - |
| Ziveh (37)                 |        |              | 29                     | 18 2 1 0 2 0 4 2 0 | 3 2 4 7 2 - |
| Nazlu (39)                 |        |              | 30                     | 3 0 3 6 0 2 11 8 | 3 6 13 5 25 |
| Gojar (25)                 |        |              | 17                     | 8 1 3 0 0 0 5 9 | 3 0 5 8 9 |
| Zangalan (21)              | Summer | 1            | 16                     | 4 7 0 0 0 0 0 5 1 | 1 0 5 5 11 |
| Nazlu (18)                 |        |              | 13                     | 3 3 0 2 0 3 0 2 6 | 2 3 2 6 7 |
| Balu (27)                  |        |              | 21                     | 7 9 0 1 0 2 0 2 | 16 1 2 2 |
| Total                      |        |              | 16                     | 246 108 37 13 11 | 15 16 2 44 |

Notes: *month; **year; D = Diarrheic (soft to diarrheic and or watery feces); ND = Non-diarrheic (normal to semi soft feces); F = Female; M= male; Mo = Mountainous; Ms = Mountain side; P = plain.

Table 2. Prevalence (%) of cattle Cryptosporidium infection (n = 246) and individual Cryptosporidium species from Urmia suburban of WAP, Iran.

| Study area (No. of cattle) | Season | Prevalence (n/N) | Fecal consistency | Age (year) | Sex (%) | Cryptosporidium (%) |
|----------------------------|--------|------------------|-------------------|------------|---------|--------------------|
|                            |        |                  | <6*               | >6 - 3**   | 4 - 6   | >6 | <6 | >6 | 4 - 6 | >6 | M | F | Cp | Ca |
| Nushan (12)                | Fall   | 0                | 0 0 0 0 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 |
| Daresanji (8)              |        | 0                | 0 0 0 0 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 |
| Band (16)                  |        | 12               | 0 8 0 0 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 |
| Nushan (19)                | Winter | 0                | 0 0 0 0 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 |
| Daresanji (21)             |        | 16               | 0 8 0 0 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 |
| Band (23)                  |        | 36               | 0 0 0 0 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 |
| Ghasebani (20)             |        | 52               | 0 0 0 0 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 |
| Bardasur (29)              |        | 2.0              | 0 0 0 0 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 |
| Gharaghaj (33)             |        | 4.0              | 0 0 0 0 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 |
| Imamkandi (27)             |        | 3.6              | 0 0 0 0 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 |
| Ziveh (37)                 |        | 5.2              | 0 0 0 0 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 |
| Nazlu (39)                 |        | 2.0              | 0 0 0 0 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 |
| Gojar (25)                 |        | 1.6              | 0 0 0 0 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 |
| Zangalan (21)              |        | 1.4              | 0 0 0 0 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 |
| Nazlu (18)                 |        | 2.0              | 0 0 0 0 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 |
| Balu (27)                  |        | 2.8              | 0 0 0 0 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 | 0 0 0 0 0 |
| Total                      |        | 134.0b           | 0 0 0 0 0 0 0 0 0 | 17.0sc     | 2.4 12 2 | 10.7sc 10.5 | 20.3 2.0 |

Notes: *month; **year; Ca = Cryptosporidium andersoni; Cp = Cryptosporidium parvum; n = Number of animals infected with Cryptosporidium; N = Total number of examined animals; NS = Non-significant; S = Significant. a (p < 0.05); b (p = 0.025); c (p < 0.0001); d (p = 0.026).
and winter ($p < 0.01$). These trends may reflect direct zoonotic contact and indirect effects of rainfall, farming events such as calving, and environmental pollution with farm waste.

In the present study, the age of examined cattle had significant effect on the prevalence. In addition, the prevalence of *C. parvum* was decreased with increasing age. The age related distribution of *C. parvum* infection in this age group was similar to that previously reported in cattle of other parts of Iran. Cryptosporidium parvum has been also reported to primarily infect D young calves and shed the specie. It seems that ND older cattle with low prevalence and without clinical symptoms of cryptosporidiosis may serve as carriers for young calves with an immature immune system in the region. Cryptosporidium infection in this study was considered to be a probable cause of diarrhea in neonates as significant association was found in previous studies. Fotouhi Ardekani *et al.* found significant difference between D (31.8%) and ND (17.4%) conditions. Also, Brook *et al.* noted that age was correlated with consistency of the feces so that in younger animals, feces are tending to be looser due to the liquid nature of the milk diet. Sex of examined cattle in the present study had also significant association with the prevalence. This finding was in concordance with previous research by Radfar *et al.*

The findings described in this investigation suggested that *C. parvum* is the most common species in cattle and farms should be also considered as a potential source of surface water contamination. Thus, further investigations may reveal more information about economic effects of this parasite and public health concern in the region. Furthermore the source of infection should be investigated and control measures should be established in the future.

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**References**

1. Yakhchali M, Moradi T. Prevalence of Cryptosporidium infection in one-humped camels (*Camelus dromedarius*) of northwestern Iran. Parasite 2012; 19(1): 71-75.
2. Fayer R. Cryptosporidium and cryptosporidiosis. Boca Raton, USA: CRC Press 1997; 1-41.
3. Sohrabi Haghdust A. The first report of gastric cryptosporidiosis in cattle of Iran. J Vet Med Fac Univ Tehran 1992; 47(1-2): 51-59.
4. Azizi HR, Purjafar M, Dabaghzadeh B, et al. A survey on prevalence of Cryptosporidium parvum in dairy cattle of Shahrekord, Iran. Iran Vet J 2007; 3(4): 96-98.
5. Hamedi Y, Safa O, Haydari M. Cryptosporidium infection in diarrheic children in southeastern Iran. J Pediatr Infect Dis J 2005; 24: 86-88.
6. Razavi SM, Oryan A, Bahrami S, et al. Prevalence of Cryptosporidium infection in camels (*Camelus dromedarius*) in a slaughterhouse in Iran. Trop Biomed 2009; 26(3): 267-273.
7. Yakhchali M, Gholami E. Prevalence of Eimeria and Cryptosporidium spp in cattle of Sanandaj city (Kurdistan province), Iran. Pajouhesh and Sazandegi 2008; 87: 81-87.
8. Fayer R. Taxonomy and species delimitation in Cryptosporidium. Exp Parasitol 2010; 124: 90-97.
9. McLaughlin J, Amar C, Pedraza-Diaz S, et al. Molecular epidemiological analysis of Cryptosporidium spp. in the United Kingdom: Results of genotyping Cryptosporidium spp. in 1705 fecal samples from humans and 105 fecal samples from livestock animals. J Clin Microbiol 2000; 38: 84-90.
10. Xiao L, Sulaiman IM, Ryan UM, et al. Host adaptation and host-parasite co-evolution in Cryptosporidium: Implications for taxonomy and public health. Int J Parasitol 2002; 32: 1773-1785.
11. Pirestani M, Sadraei J, Dalimi Asl A, et al. Molecular characterization of Cryptosporidium isolates from human and bovine using 18S rRNA gene in Shirahri county of Tehran, Iran. Parasitol Res 2008; 103(2): 447-457.
12. Olson ME, O’Handley RM, Ralston BJ, et al. Update on Cryptosporidium and Giardia infections in cattle. Trends Parasitol 2004; 20(4): 185-191.
13. Dorostkar Moghadam D, Azami M, Salehi R, et al. Cryptosporidium spp. detection by using 18S rRNA gene and PCR-RFLP. Iran J Basic Med Sci 2005; 8(4): 232-238.
14. Fotouhi Ardekani R, Fashi Harandi M, Banai S, et al. Epidemiology of Cryptosporidium infection in Kerman/Iran and Molecular genotyping of some isolates. J Kerman Uni Med Sci 2008; 15(4): 313-320.
15. Iranian Veterinary Organization Web site. Population of domestic animals of Iran. Available at: http://www.ivo.org.ir/portal. Accessed Nov 26, 2012.
16. Smallwood JE. A guide tour of veterinary anatomy: Domestic ungulates and laboratory mammals. Philadelphia, USA: WB Saunders 1992; 322-323.
17. Thrusfield M. Veterinary Epidemiology. 3rd ed. New Jersey, USA: Blackwell Science 2005; 233.
18. Henrickson SA, Polenz JF. Staining of Cryptosporidia by a modified Ziehl-Neelsen technique. Acta Vet Scand 1981; 22: 594-596.
19. Soulsby EJL. Helminths, Arthropods and Protozoa of Domesticated Animals. 7th ed. London, UK: Bailliere Tindall 1982; 607, 612-613.
20. Arrowood MJ, Sterling CR. Isolation of Cryptosporidium oocysts and sporozoites using discontinuous sucrose and Isopycnic Percoll gradients. J Parasitol 1987; 73(2): 314-319.
21. Karanis P, Plutzer J, Halim N, et al. Molecular characterization of Cryptosporidium species from animal sources in Qinghai province of China. Parasitol Res 2007; 101(6): 1575-1580.
22. Sambrook J, Russell DW. Molecular cloning: A laboratory manual. 3rd ed. New York, USA: Cold Spring Harbor Laboratory Press 2002; 122-125.
23. Xiao L, Escalante L, Yang C, et al. Phylogenetic analysis of Cryptosporidium parasites based on the small-subunit rRNA gene locus. Appl Environ Microbiol 1999; 65(4): 1578-1583.
24. Lindsay DS, Upton SJ, Owens DS, et al. Cryptosporidium andersoni n. sp. (Apicomplexa: Cryptosporidiidae) from cattle, Bos taurus. J Eukaryot Microbiol 2000; 47(1): 91-95.
25. Nguyen ST, Nguyen DT, Le DQ, et al. Prevalence and first genetic identification of Cryptosporidium sp. in cattle in central Vietnam. Vet Parasitol 2007; 150(4):357-361.
26. Santin M, Trout JM, Xiao L, et al. Prevalence and age-related variation of Cryptosporidium species and genotypes in dairy calves. Vet Parasitol 2004; 122(2):103-117.
27. Slapeta J. Cryptosporidium species found in cattle: A proposal for a new species. Trends Parasitol 2006; 22(10): 469-474.
28. 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 2007; 145(3-4): 260-266.
29. Xiao LH, Fayer R, Ryan U, et al. Cryptosporidium taxonomy: Recent advances and implications for public health. Clin Microbiol Rev 2004; 17(1): 72-97.
30. Brook E, Anthony Hart C, French N, et al. Prevalence and risk factors for Cryptosporidium spp. infection in young calves. Vet Parasitol 2008; 152(1-2): 46-52.
31. Azami M. Prevalence of Cryptosporidium infection in cattle in Isfahan, Iran. J Eukaryot Microbiol 2007; 54(1): 100-102.
32. Radfar MH, Molaei MM, Baghbannejad A. Prevalence of Cryptosporidium spp. Oocysts in dairy calves in Kerman, southeastern Iran. Iran J Vet Res 2006; 7(2): 81-84.
33. Sevinç F, İrmak K, Sevinç M. The prevalence of Cryptosporidium parvum infection in the diarrhoeic and non-diarrhoeic calves. Rev Med Vet-Toulouse 2003; 154: 357-361.
34. Seuli SR, Samar S, Subhasis B, et al. Observations on the epidemiology of bovine cryptosporidiosis in India. Vet Parasitol 2006; 141(3-4): 330-333.
35. Geurden T, Goma FY, Siwila J, et al. Prevalence and geno-typing of Cryptosporidium in three cattle husbandry systems in Zambia. Vet Parasitol 2006; 138(3-4): 217-222.
36. Singh BB, Sharma R, Kumar H, et al. Prevalence of Cryptosporidium parvum infection in Punjab (India) and its association with diarrhea in neonatal dairy calves. Vet Parasitol 2006; 140(1-2), 162-165.