Research Journal of Parasitology

ISSN 1816-4943

Academic Journals Inc.

www.academicjournals.com

Research Journal of Parasitology 6 (3): 101-108, 2011
ISSN 1816-4943 / DOI: 10.3923/jp.2011.101.108

Prevalence and Molecular Discrimination of *Cryptosporidium parvum* in Calves in Behira Provinces, Egypt

[1]M.A. Hassanain, [2]Fathia A.M. Khalil, [3]K.A. AbdEl-Razik and [1]R.M. Shaapan
[1]Department of Zoonosis, Veterinary Research Division, National Research Center, Dokki, Giza, Egypt
[2]Department of Parasitology and Animal Diseases, National Research Center, Dokki, Giza, Egypt
[3]Department of Animal Reproduction, National Research Center, Dokki, Giza, Egypt

Corresponding Author: Raafat M. Shaapan, Post Box 12622, El-Tahrir Street, Dokki, Giza, Egypt Tel: 00202-25272439, 002-0105280571 Fax: 00202-33371362

ABSTRACT

Cryptosporidium parvum is a ubiquitous zonootic protozoan parasite which is associated with severe acute diarrhoea in humans and animals. Diarrhoiec fecal samples collected from young cattle calves at different localites in Behira Province, Egypt were coproscopically examined concerning the presence of *Cryptosporidium* spp., oocysts. Small sized *Cryptosporidium* oocysts were detected of typical shape and measurements of the *C. parvum* oocysts with a total prevalence of 54.4% (higher prevalence 61.6% in calves less than 1 month of age while lower 38.2% in calves aged 1-2 month). Molecular characterization was done using nested PCR amplification and partial sequence analysis. The nested PCR gave the expected 1st (1325 bp) and the 2nd (825 bp) PCR products from all the examined Egyptian isolates. The DNA sequence alignments and BLAST search analysis of the of Egyptian isolates of *Cryptosporidium* revealed 100% homology between the 825 bp amplified fragment of Egyptian isolates and the counterpart of the 18S rDNA sequences of *C. parvum* deposited in Gene bank and proved that the 10 positive PCR isolates were *Cryptosporidium parvum*. The high prevalence of *Cryptosporidium parvum* among calves obtained by this investigation has pointed to the existence of this zoonotic genotype and suggesting that there is a potential risk of Cryptosporidiosis as a zoonotic transmission between calves and humans in this region.

Key words: *Cryptosporidium parvum,* calves, coproscopical examination, nested PCR analysis, DNA sequences

INTRODUCTION

The protozoan parasites *Cryptosporidium* are widespread in the environment have proven potential to cause waterborne and foodborne disease and the major outbreaks of cryptosporidiosis have occurred as a result of contaminated drinking water. Their entire life cycle takes place in the intestinal epithelium and transmission occurs in the form of environmentally resistant oocysts by the fecal-oral route and/or through contaminated food and/or water (Dawson, 2005; Smith and Nichols, 2010).

The genus *Cryptosporidium* is composed of at least 13 species. Cattle are infected with at least 4 Cryptosporidium parasites: *C. parvum, C. bovis, C. andersoni* and the *C. ryanae*. The occurrence of these *Cryptosporidium* spp., in cattle were shown to be age-related and *C. parvum* is the only prevalent zoonotic species in cattle, is responsible for about 85% of the *Cryptosporidium* infections

in pre-weaned calves but only 1% of the *Cryptosporidium* infections in postweaned calves and 1-2-year-old heifers (Santin *et al*., 2004; Fayer *et al*., 2006).

Cryptosporidium parvum is the important species which infects the gastrointestinal epithelium of most mammalian hosts including cattle and human worldwide, causes severe acute diarrheal enteritis with substantial morbidity and mortality among young calves, children and immunocompromised individuals such as AIDS patients (Chalmers and Davies, 2010). Contact with infected calves has been implicated as the cause of many cryptosporidiosis outbreaks in veterinary students, research technicians and children attending agricultural (Coco *et al*., 2009).

Extra-intestinal infections by *Cryptosporidium parvum* have been detected in pigs and sheep. In addition to intestinal infections, cryptosporidial oocysts were found in the gall-bladders of two pigs which were 2 months old and in some organs of sheep aged 5 days, including the gall-bladder, mesenteric lymph nodes, trachea, lung and the uterus of one lamb as reported by Fleta *et al*. (1995). Moreover, infection of the uterine mucosa was induced after experimental intra-uterine inoculation of adult mice with *C. parvum* (Liebler *et al*., 1986).

Lack of distinctive morphologic features of Cryptosporidium oocysts render microscopical examination inconvenient to clearly differentiate species rather genotypes (Fall *et al*., 2003). Therefore, molecular analyses have been widely used to characterize the genetic structure of *Cryptosporidium* parasites and assessment of their zoonotic significance (Xiao, 2010). Molecular analysis of *C. parvum* revealed several families among isolates from humans and/or cattle as well as several subtypes within each family (Wielinga *et al*., 2008). Consequantelly, *C. parvum* can be divided into two genetically distinct subpopulations based on the molecular characterization of oocysts: genotype I (anthroponotic genotype) which is associated exclusively with human infection and is recently proposed as a new species, *C. hominis* and genotype II (zoonotic or cattle genotype) which is associated with both human and animal infections (Hajdusek *et al*., 2004; Abe *et al*., 2006).

In Egypt, limited studies were devoted to ascertain cryptosporidiosis in human and animals. However, Cryptosporidium was responsible for considerable part of diarrheal illness among American military personnel participated in a military exercise in the northwestern Egyptian desert (Sanders *et al*., 2005). Human cryptosporidiosis was also reported in different ages (Youssef *et al*., 2008). *Cryptosporidium* infection frequently affects domestic animals with strong relation between *C. parvum* infection and diarrhoea among Egyptian buffalo calves (Warda *et al*., 2002; El-Khodery and Osman, 2008). Recentally, epidemiological and molecular findings on *Cryptosporidium* spp., from Egyptian dairy calves and stray dogs were performed by Amer *et al*. (2010) and El-Madawy *et al*. (2010).

Though cryptosporidiosis is frequent in human and livestock in Egypt, very little information is available about the genetic diversity of this parasite. Therefore, this study was aimed to determine the prevalence and as well as molecular heterogeneity of *Cryptosporidium* spp., isolated from calves in Behira Province, Egypt. To this end, species identification was based on nested PCR amplification and DNA sequence analysis of these Egyptian isolates and compared with counterparts deposited in Gene bank.

MATERIALS AND METHODS

Coproscopical examination

Fecal samples: A total of 180 diarrhoeic fecal samples were collected from young cattle calves (125 from less than 1 month and 55 from 1-2 months-aged calves) in different localites at Behira Province, Egypt.

Detection of oocysts: Fine feces smears fixated with methanol spirit and stained with Modified Ziehl-Neelsen Stain (MZN) for detection of *Cryptosporidian* oocysts according to the procedure described by Henriksen and Pohlenz (1981). The preparations were observed and the oocysts were measured with the help of stage micrometer conjugated with a light microscope at the eyepiece 10x and the objective 100x. All measurements were taken in micrometers (μm) for about 20-50 oocysts, with the range in parenthesis following the mean (Fayer and Xiao, 2008).

POLYMERASE CHAIN REACTION (PCR)

PCR-sample preparation: Oocysts of *Cryptosporidium* were purified from collected calve fecal samples following sucrose and Percoll flotation (Arrowood and Sterling, 1987). The purified oocysts were washed three times with PBS solution and then stored at -20°C in 2.5% potassium dichromate solutions.

DNA extraction: DNA was extracted from the washed *Cryptosporidium* oocysts using the DNeasy Blood and Tissue Kit (Qiagen Co., Cat. no. 69504) with modifications to the manufacturer's protocols according to Lalonde and Gajadhar (2009) as follows : (i) oocysts were first suspended in 300 mL of ATL buffer and subjected to 8 cycles of freeze-thaw for 1 min in liquid nitrogen and 1 min in a 95°C water bath; (ii) lysed suspensions were incubated with 20 mL proteinase K (20 mg mL^{-1}, Qiagen) for 3 h at 56°C, followed by incubation at 70°C for 10 min in 300 mL AL buffer with vortexing for 10 sec every 3 min. DNA was purified through the provided columns according to the manufacturer's protocol and eluted in 50 mL of the supplied AE buffer and stored at -20°C.

Nested PCR procedure: For the primary PCR (expected amplicon size: 1325 bp), primers were used as described by Xiao *et al.* (2004): 18 SF: 5′-TTCTAGAGCTAATACATGCG-3′ (forward) and 18 SR: 5′-CCCATTTCC TTCGAAACAGGA-3′ (reverse). For the secondary PCR (expected amplicon size: 819-825 bp, depending on the species), the following primers were used: 18 SNF: 5′-G G A A G G G T T G T A T T T ATTAGATAAAG-3′ (forward) and 18 SNR 5′-AAGG A G T A A G G A A CAACCTCCA-3′ (reverse). The primary Polymerase Chain Reaction (PCR) contained: 1 μL (50 pmol) of each primer (18SF and 18SR), 28 μL Taq PCR Master Mix (Qiagen Co. Cat. no. 201443) and 3 μL of DNA sample and milliQ water up to a total volume of 50 mL. The conditions for the secondary PCR were the same as for the primary PCR. Both procedures were performed in a thermocycler (PTC-100 Thermal Cycler, MJ Research, Incline Village, USA) starting with initial denaturation of 95°C/2 min with the following cycles: 35 cycles of 94°C/45 sec, 62°C/45 sec, 72°C/1 min^{-1}, with a final extension of 72°C/10 min. PCR products were separated on 1.2% agarose gels and visualized after ethidium bromide staining.

DNA sequencing: PCR products were sequenced in a commercial sequencing laboratory (Fermentas GMBH, Germany). The sequences obtained were submitted to BLAST (Basic Local Alignment Search Tool) (Altschul *et al.*, 1997) in order to identify their similarity with sequences of the GenBank. Sequence alignment was performed using CLUSTAL W (Thompson *et al.*, 1994) with manual adjustments.

RESULTS

Morphology and measurements of *Cryptosporidium* oocysts: The detected *Cryptosporidium* oocysts in the examined calve feces stained with Ziehl-Nelson technique were morphologically

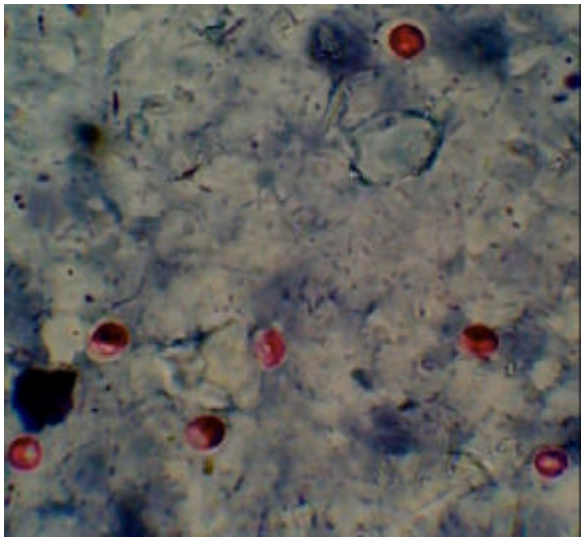

Fig. 1: *Cryptosporidium parvum* oocysts in calve fecal smears stained with Modified Ziehl-Neelsen stain (MZN) (X100)

Table 1: Dimensions of *Cryptosporidium parvum* oocysts detected in calve fecal samples

Parameters	Dimensions of the *Cryptosporidium parvum* oocysts (µm)
Length×width	(4.4-5.8)×(4.3-4.9)
Mean	5.1×4.6
Shape Index (SI) l/w	1.0-1.2 (1.1)

Table 2: Prevalence of *Cryptosporidium parvum* infection among calves

Age	Examined No.	Positive No.	Positive (%)
Less than 1 month calves	125	77	61.6
1-2 month calves	55	21	38.2
Total calves	180	98	54.4

similar to *Cryptosporidium parvum* oocysts which characterized by spherical to ovoid shape with smooth wall and appeared as acid fast (red-pink) on green back. The measurements of 50 oocysts were varied from 4.4-5.8×4.3-4.9 µm of mean (5.1×4.6) and the shape index was 1.0-1.2 of mean (1.1) (Fig. 1 and Table 1).

Prevalence of *Cryptosporidium* oocysts: The extent of prevalence was 54.4% (98 out of 180) of total diarrhoeic fecal calve samples were found to be infected with *Cryptosporidium parvum* oocysts. The older (1-2 month) calves revealed lower prevalence 38.2% (21 out of 55) while the younger (less than 1 month) calves showed higher *Cryptosporidium* infection 61.6% (77 out of 125) (Table 2).

Nested PCR analysis: Using DNA extracted directly with simple modification from *Cryptosporidium* oocysts in nested PCR. This technique showed high sensitivity and specificity in the diagnosis of *Cryptosporidium* spp., in ruminants. The nested PCR gave the expected 1st (1325 bp) and the 2nd (825 bp) PCR products from all the examined Egyptian isolates (Fig. 2).

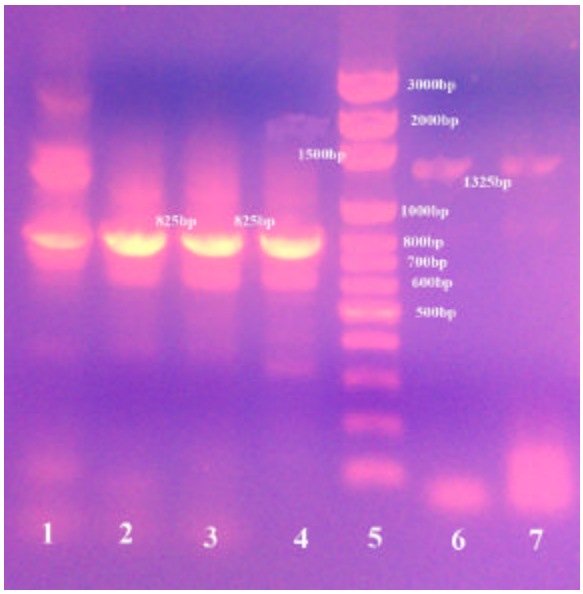

Fig. 2: Nested PCR of *Cryptosporidium* DNA: Lane 1-3, shows 2nd PCR products (825 bp) from *Cryptosporidium* DNA; Lane 5, molecular DNA size markers; Lanes 4 and 6, Positive controls (*Cryptosporidium parvum* DNA) and Lane 7, 1st PCR product (1325 bp) from *Cryptosporidium* DNA

DNA sequencing: The nucleotide sequence alignments and BLAST search analysis of the Egyptian isolates of *Cryptosporidium* in GenBank revealed 100% homology between the 825 bp amplified fragment of Egyptian isolates and the counterpart of the 18S rDNA sequences of *C. parvum*. Homology search proved that all the 10 isolates were *Cryptosporidium parvum*.

DISCUSSION

The *Cryptosporidium* oocysts detected in calve fecal smears in this study are morphologically similar to those of *Cryptosporidium parvum* described in calves in many previous studies (Fall *et al*., 2003; Fayer *et al*., 2006; Quilez *et al*., 2008). Identification of the *Cryptosporidium* species was depending upon the conventional criteria, such as oocyst morphology and measurements, this opinion was agreed with Fayer *et al*. (2000) and Morgan-Ryan *et al*. (2002), who cited that morphometric measurement of oocysts represents the cornerstone of *cryptosporidium* taxonomy and is one of the requirements for establishing a new species, however, is not adequate by itself and should using multiple parameters as electron microscopy, developmental biology, host specificity, histopathology and/or molecular biology.

In the present study, 98 out of 180 (54.4%) of the total investigated calves were confirmed by acid fast stain to be infected with *Cryptosporidium*. These finding was higher than (21.7 and 30.2%) detected by Amer *et al*. (2010) and El-Khodery and Osman (2008) in Egyptian buffalo calves and dairy calves, respectively; but lower than (95%) estimated by Ramirez *et al*. (2004) in US dairy farms; wherease, these results are consistent with (52%) had been reported by Silverlas *et al*. (2009) in Europian young calves. Several factors may be responsible for the differences in the prevalence from the present study to those previously surveyed, such as the breed of the calves, husbandry and management system, exac age of the host, nursing conditions of the calves, season of the sample collection as well as the sanitary conditions inside and around the

farms. Some of these factors may act individually or collectively to increase the risk factor associated with transmission and prevalence of *Cryptosporidium* between calves (Kvac *et al*., 2006; Duranti *et al*., 2009).

Concerning age, higher prevalence of cryptosporidiosis in our study (61.6%) was detected in calves less than 1 month however lower prevalence (38.2%) in 1-2 month aged calves. These findings agreed with Fayer *et al*. (2006) in the United States who demonstrated that only preweaned calves are important sources of zoonotic cryptosporidiosis in humans and also Kvac *et al*. (2006) and Xiao (2010) reported that, calves less than 2 months of age are the major contributors of zoonotic *C. parvum*.

It is crucial to differentiate among *Cryptosporidium* species infecting cattle because only *C. parvum* is zoonotic type of *C. parvum* and is known to be infectious to many mammalian hosts worldwide, where *C. bovis*, *C. andersoni* and *C. ryanae* are cattle adapted (Fayer *et al*., 2006; Santin and Zarlenga, 2009). Using nested PCR and sequences of the rDNA, this study revealed that calves, in Behira Province, Egypt, are only infected with the zoonotic *C. parvum* assumed to be of the cattle genotype. Such genotype is detected among animals worldwide (Fayer *et al*., 2000; Hajdusek *et al*., 2004; Abe *et al*., 2006). In Egypt, DNA amplification of *C. parvum* has been identified only in calves (Warda *et al*., 2002). However, little information on gene sequence of isolates from *Cryptosporidium* species associated with animal hosts in Egypt has been reported so, extensive studies are extremely important including biological aspects associated with molecular techniques.

Regarding the epidemiology of *C. parvum* bovine genotype, water-, food-, or air-borne transmission and person-toperson or animal-human contacts are reported routes of infection, with a median dose of 130 oocysts (Fayer *et al*., 2000). In developing countries, the risk of infection may be higher and the disease probably exerts most of its impact on neonates and infants and raditionally, cattle have been considered the primary non-human species impacted by cryptosporidiosis and the major reservoir of *Cryptosporidium* for human infections (Hunter *et al*., 2009). The animal-human contact is considered as the most important transmission route in Egypt, with cattle probably representing the most important source of human and animal infections (Warda *et al*., 2002).

CONCLUSION

Calves in Egypt carrying the zoonotic *C. parvum* could represent the important farm animal source of infection to human. The high *Cryptosporidium* prevalence rates among calves obtained by this study indicate the potential risk of its transmission to humans through contact with infected cattle feces or contaminated milk or food with these oocysts. Further molecular analyses are needed to elucidate the nature of the other unknown genotypes of *Cryptosporidium* sp., to better understand the source of infection and routes of transmission and ultimately, to improve risk assessment and measures for prevention and control of cryptosporidiosis

ACKNOWLEDGMENTS

The authors would like to express their gratitude to Dr. Hesham Abdeen (Belfast Health and Social care Trust, Belfast, UK) for his assistance in carrying out the sequence analysis of PCR products.

REFERENCES

Abe, N., M. Matsubayashi, I. Kimata and M. Iseki, 2006. Subgenotype analysis of *Cryptosporidium parvum* isolates from humans and animals in Japan using the 60-kDa glycoprotein gene sequences. Parasitol. Res., 99: 303-305.

Altschul, S.F., T.L. Madden, A.A. Schaffer, J. Zhang, Z. Zhang, W. Miller and D.J. Lipman, 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucl. Acids Res., 25: 3389-3402.

Amer, S., H. Honma, M. Ikarashi, C. Tada, Y. Fukuda, Y. Suyama and Y. Nakai, 2010. Cryptosporidium genotypes and subtypes in dairy calves in Egypt. Vet. Parasitol., 169: 382-386.

Arrowood, M.J. and C.R. Sterling, 1987. Isolation of *Cryptosporidium* oocysts and sporozoites using discontinuous sucrose and isopycnic Percoll gradients. J. Parasitol., 73: 314-319.

Chalmers, R.M. and A.P. Davies, 2010. Minireview: Clinical cryptosporidiosis. Exp. Parasitol., 124: 138-146.

Coco, D.V.F., M.A. Cordoba and J.A. Basualdo, 2009. Cryptosporidiosis: An emerging zoonosis. Rev. Argent Microbiol., 41: 185-196.

Dawson, D., 2005. Foodborne protozoan parasites. Int. J. Food Microbiol., 103: 207-227.

Duranti, A., S.M. Caccio, E. Pozio, A. di Egidio, M. de Curtis, A. Battisti and P. Scaramozzino, 2009. Risk factors associated with *Cryptosporidium parvum* infection in cattle. Zoonoses Public Health, 56: 176-182.

El-Khodery, S.A. and S.A. Osman, 2008. Cryptosporidiosis in buffalo calves (*Bubalus bubalis*): Prevalence and potential risk factors. Trop. Anim. Health Prod., 40: 419-426.

El-Madawy, R.S., N.O. Khalifa and H.F. Khater, 2010. Detection of cryptosporidial infection among Egyptian stry dogs by using *Crypyosopridium parvum* wall protein gene. Bulgarian J. Vet. Med., 13: 104-110.

Fall, A., R.C.A Thompson, R.P. Hobbs and U. Morgan-Ryan, 2003. Morphology is not a reliable tool for delineating species within *Cryptosporidium*. J. Parasitol., 89: 399-402.

Fayer, R. and L. Xiao, 2008. *Cryptosporidium* and *Cryptosporidiosis*. 2nd Edn., CRC Press/Taylor and Francis Group, Boca Raton, FL., ISBN-13: 9781420052268, USA, Pages: 560.

Fayer, R., M. Santin, J.M. Trout and E. Greiner, 2006. Prevalence of species and genotypes of *Cryptosporidium* found in 1-2- years old dairy cattle in the eastern United States. Vet. Parasitol., 135: 105-112.

Fayer, R., U. Morgan and S.J. Upton, 2000. Epidemiology of *Cryptosporidium*: Transmission, detection and identification. Int. J. Parasitol., 30: 1305-1322.

Fleta, J., C. Sanchez-Achedo, A. Clavel and J. Quilez, 1995. Detection of *Cryptosporidium* oocysts in extra-intestinal tissues of sheep and pigs. Vet. Parasitol., 59: 201-205.

Hajdusek, O., O. Ditrich and J. Slapeta, 2004. Molecular identification of *Cryptosporidium* spp., in animal and human hosts from the Czech Republic. Vet. Parasitol., 122: 183-192.

Henriksen, S.A. and J.F. Pohlenz, 1981. Staining of Cryptosporidia by a modified Ziehl-Neelsen technique. Acta Vet. Scand., 22: 594-596.

Hunter, P., D. Zmirou-Navier and P. Hartemann, 2009. Estimating the impact on health of poor reliability of drinking water interventions in developing countries. Sci. Total Environ., 407: 2621-2624.

Kvac, M., M. Kouba and J. Výtovec, 2006. Age-related and housing dependence of *Cryptosporidium* infection of calves from dairy and beef herds in South Bohemia, Czech Republic. Vet. Parasitol., 137: 202-209.

Lalonde, L.F. and A.A. Gajadhar, 2009. Effect of storage media, temperature and time on preservation of *Cryptosporidium parvum* oocysts for PCR analysis. Vet Parasitol., 160: 185-189.

Liebler, E.M., J.F. Pohlenz and D.B. Woodmansee, 1986. Experimental intra-uterine infection of adult BABL/ c mice with *Cryptosporidium* sp. Infect. Immun., 57: 255-259.

Morgan-Ryan, U.M., A. Fall, L.A. Ward, N. Hijjawi and I.M. Sulaiman *et al.*, 2002. *Cryptosporidium hominis* n. sp., (Apicomplexa: Cryptosporidiidae) from *Homo sapiens*. J. Eukaryot Microbiol., 49: 433-440.

Quilez, J., E. Torres, R.M. Chalmers, G. Robinson, E.D. Cacho and C. Sanchez-Acedo, 2008. *Cryptosporidium* species and subtype analysis from dairy calves in Spain. Parasitology, 135: 1613-1620.

Ramirez, N.E., L.A. Ward and S.A. Sreevatsan, 2004. Review of biology and epidemiology of cryptosporidiosis in humans and animals. Microbiol. Infect., 6: 773-785.

Sanders, J.W., S.D. Putnam, P. Gould, J. Kolisnyk and N. Merced *et al.*, 2005. Diarrheal illness among deployed U.S. military personnel during Operation Bright Star 2001-Egypt. Diagn. Microbiol. Infect. Dis., 52: 85-90.

Sanders, J.W., S.D. Putnam, M.S. Riddle and D.R. Tribble, 2005. Military importance of diarrhea: Lessons from the Middle East. Military importance of diarrhea: Lessons from the Middle East. 21: 9-14.

Santin, M., J.M. Trout, L. Xiao, L. Zhou, E. Greiner and R. Fayer, 2004. Prevalence and age-related variation of *Cryptosporidium* species and genotypes in dairy calves. Vet. Parasitol., 122: 103-117.

Santin, M. and D.S. Zarlenga, 2009. A multiplex polymerase chain reaction assay to simultaneously distinguish *Cryptosporidium* species of veterinary and public health concern in cattle. Vet. Parasitol., 166: 32-37.

Silverlas, C., U. Emanuelson, K. de Verdier and C. Bjorkman, 2009. Prevalence and associated management factors of *Cryptosporidium* shedding in 50 Swedish dairy herds. Prev. Vet. Med., 90: 242-253.

Smith, H.V., and R.A. Nichols, 2010. *Cryptosporidium*: Detection in water and food. Exp. Parasitol., 124: 61-79.

Thompson, J.D., D.G. Higgins and T.J. Gibson, 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res., 22: 4673-4680.

Warda, M., A. El-Ghaysh, M. Ghoneim, F. Khalil and M. Hilali, 2002. Polymerase chain reaction is a detectable tool to discriminate between *Cryptosporidium parvumand* other apicomplexan parasites using C. parvum 18s RRNa and Outer Wall Protein (COWP) genes. Proceedings of the 10th scientific Conference, Faculty of Veterinary Medicine, December 2002, Assiut University, Egypt, pp: 449-457.

Wielinga, P.R., A. de Vries, T.H. van der Goot, T. Mank, M.H. Mars, L.M. Kortbeek and J.W.B. van der Giessen, 2008. Molecular epidemiology of Cryptosporidium in humans and cattle in the Netherlands. Int. J. Parasitol., 38: 809-817.

Xiao, L., 2010. Molecular epidemiology of cryptosporidiosis: An update. Exp. Parasitol., 124: 80-89.

Xiao, L., A.A. Lal and J. Jiang, 2004. Detection and Differentiation of *Cryptosporidium* Oocysts in Water by PCR-RFLP. In: Public Health Microbiology: Methods and Protocols, John, F.T., S. Rugut and L. Alicia (Eds.). Humana Press, Otowa, pp: 163-176.

Youssef, F., I. Adib, M. Riddle and C. Schlett, 2008. A review of cryptosporidiosis in Egypt. J. Egypt. Soc. Parasitol., 38: 9-28.