

## Epidemiological aspects of cryptosporidiosis in Beni-Suef Governorate

M. A. Ibrahim<sup>1</sup>, S. M. Abo El-Hadid<sup>2</sup>, M. H. Meabed<sup>3</sup>

<sup>1</sup>Department of Hygiene, Management and Zoonoses, Faculty of Veterinary Medicine Beni-Suef University, Beni-Suef, Egypt

<sup>2</sup>Department of Parasitology, Faculty of Veterinary Medicine Beni-Suef University, Beni-Suef, Egypt

<sup>3</sup>Department of Pediatrics, Faculty of Medicine, Beni-Suef University, Beni-Suef, Egypt

The aim of this study was to evaluate the status of infection by *Cryptosporidium* spp. in Beni-Suef Governorate. For this purpose, 305 fecal samples from animals (75 calves, 74 dairy cattle, 86 lambs and 70 sheep) and 177 human stool samples were collected from inhabitants and out patients of the university hospital in Beni-Suef who suffering from gastroenteritis mainly diarrhea. The samples were examined using Modified Ziehl-Neelsen technique. An over all of animal cryptosporidiosis was 6.8%. *Cryptosporidium* oocysts could be detected in calves, dairy cattle, lambs and adult sheep at a rate of 12%, 2.75%, 6.97% and 0% respectively. Concerning humans *Cryptosporidium* oocysts could be detected at a rate of 14.56% and 2.7% in diarrheic individuals and apparently healthy ones. Infection rate in females (14.28%) was higher than in males (6%) and the children (1-10 years old) were highly susceptible to cryptosporidiosis than adults. Eighty-nine selected diarrheic fecal samples (79 from humans and 10 from calves) were selected and examined by cryptosporidium antigen capture ELISA beside their examination by Modified Ziehl-Neelsen technique. No significant differences were found between the two techniques in terms of epidemiological study.

Cryptosporidiosis is a protozoan infection caused by *Cryptosporidium* species. The protozoon colonizes epithelial cells of the gastrointestinal tract of vertebrates causing enteric diseases in man and animals (Janoff and Reller 1987).

The genus *Cryptosporidium* contains several species that were identified in a vast sort of hosts including mammals, birds, fishes and reptiles (Janoff and Reller 1987). Although there are nineteen species described (Levine, 1984) so far, only *Cryptosporidium muris* and *Cryptosporidium parvum* are associated with mammals (Fayer and Ungar 1986). *Cryptosporidium parvum* is generally regarded as the major species responsible for clinical disease in humans and domestic animals (Tzipori and Ward 2002). Nevertheless, difficulties in the species identification through the use of immunologic tests and molecular biology for positive confirmation, so far, existing species, the term *Cryptosporidium* spp. has the preference when regarded to those secluded from humans (Meinhardt *et al.*, 1996).

Interest in *Cryptosporidium* by veterinary medical profession was stimulated in 1971 when this protozoon was first reported to be associated with bovine diarrhea (Pohlenz *et al.*, 1978).

Since this time, numerous case reports from different animals were now present in the literature and one species, *C. parvum*, is recognized as an important cause of the neonatal diarrhea in calves and lambs (Angus, 1983). In 1980, *Cryptosporidium* isolates obtained from calves, lambs and humans with severe diarrhea readily infected seven other species of animals (Tzipori *et al.*, 1980a). The transmission of human isolates, which induced acute diarrhea in lambs indistinguishable from that caused by other animal isolates, strongly indicated the potential zoonotic nature of *Cryptosporidium* (Tzipori *et al.*, 1980b).

The first case of human cryptosporidiosis was reported in 1976 (Nime *et al.*, 1976). Subsequent reports were rare until it was recognized that *Cryptosporidium* (*C. parvum*) may produce a short-term diarrheal illness in immunocompetent persons and a prolonged, life-threatening cholera-like illness in immunodeficient patients, especially those with AIDS (Current *et al.*, 1983 and Crawford and Vermund, 1988).

Unlike other intestinal pathogens, *Cryptosporidium* can infect several different hosts, including humans, cattle, sheep, goats, pigs and horses. It can survive in most environments for

long periods of time due to its "hardy cyst" (Keusch *et al.*, 1995) that inhabits all climates and localities.

Exposure to *C. parvum* oocysts, either directly through contact with infected humans or animals, or indirectly by drinking or eating food washed with contaminated water may lead to acute diarrhea. *C. parvum* causes an acute, self-limiting infection and diarrheal disease in immunocompetent people, in whom the onset may be rapid (3-7 days), depending on a combination of host factors (age, presence of maternal antibodies or previous exposure, and infection dose) and parasite related factors (origin and age of oocysts and species/genotype) (Tzipori and Ward, 2002). In children and immunocompromised individuals, cryptosporidiosis can be a serious, unrelenting and life-threatening problem (Blanshard *et al.*, 1992). Although the prevalence of cryptosporidiosis in people with AIDS is not high (5-15% in developed countries), the lack of effective treatment makes it one of the most troublesome among the opportunistic infections associated with AIDS.

Cryptosporidiosis was initially diagnosed in intestinal biopsy (Meisel *et al.*, 1976 and Nime *et al.*, 1976) Pohlenz, *et al.* (1978) described the finding of *Cryptosporidium* oocysts in fecal material of young calves, since then the diagnosis of this protozoan has become essentially coprologic, although it shows some limitations. Detection depends on the presence of intact oocysts in feces. The earliest method of staining of a fecal smear with modified acid fast remains the quickest and the easiest to perform if the sample is reasonably fresh although acid fast stains may fail to detect *Cryptosporidium* when being less than 100,000 oocysts/gm in formed stool or 10,000 oocyst/gm in a watery stool (Weber, *et al.*, 1991). Other methods used in clinical diagnostic laboratories include direct and indirect immunofluorescence microscopy and ELISA. These are highly sensitive techniques and are useful for screening large numbers of specimens in a short time period. That does not rely on microscopic skills.

The present study was performed to investigate the status of cryptosporidiosis infection in Beni-Suef Governorate and compare ELISA with Modified Ziehl-Neelsen technique for detection in *Cryptosporidium* oocysts in feces.

## Material and methods

**Samples from livestock.** 305 fecal samples (75 from calves, 74 from dairy cattle, 86 from lambs and 70 from adult sheep) were collected from Beni-Suef Governorate from the sites where the stool samples of humans were collected. *Cryptosporidium* oocysts were examined by modified Ziehl-Neelsen technique (Henriksen and Pohlenz, 1981).

**Samples from humans.** 177 stool samples were collected from the inhabitants residing Beni-Suef city and from out patients of the university hospital in Beni-Suef who suffering from gastroenteritis mainly diarrhea. Data concerning age and sex were recorded. Stool smears from each sample was prepared, stained by modified Ziehl-Neelsen procedure according to (Henriksen and Pohlenz, 1981) and then examined microscopically for oocysts of *Cryptosporidium*.

**Cryptosporidium antigen capture ELISA (AC ELISA).** Coproantigen of *Cryptosporidium parvum* was detected in fecal samples using *Cryptosporidium* AC-ELISA kit (R-Biopharm AG: Darmstadt, Germany, Art. No: C12010115). Eighty-nine selected stool samples (79 from diarrheic humans and 10 from diarrheic calves) were subjected to examination. Briefly, fecal samples were diluted with buffered NaCl solution 1:10 (v/v). 100µl of samples, positive and negative controls were pipetted into the wells. 100µl of HRP conjugated mouse monoclonal antibody was added to each well, mixed well and incubated for 60 min at room temperature. Wells were decanted and washed for five times with washing buffer. Then 100µl of tetramethyl-benzidine substrate working solution was added to each well and incubated for 15 min. in dark place. Reaction was stopped by adding 50µl 1 M. H<sub>2</sub>SO<sub>4</sub>. Absorbance was measured at 450 nm. ELISA results obtained were compared with those obtained by modified Ziehl-Neelsen technique.

The protozoan parasite *Cryptosporidium* causes acute gastroenteritis in both humans and animals. It has become an important pathogen all-over the world. *Cryptosporidium* spp. may cause asymptomatic or symptomatic acute infections of the digestive system in immunocompetant hosts, but life-threatening disease in immunocompromised ones. Table (1) indicated the prevalence of *Cryptosporidium* spp. among the examined livestock. *Cryptosporidium* oocysts were identified in calves, dairy cattle, lambs and sheep

## Results and Discussion

**Table (1): Prevalence of *Cryptosporidium* species among the examined animals by Modified Ziehl-Neelsen technique.**

Species of animal	No. of samples	Infected animals	
		Number	%
Calves	75	9	12
Dairy cattle	74	2	2.7
Lambs	86	6	6.97
Adult sheep	70	0	0
Total	305	17	5.57

**Table (2): Prevalence of *Cryptosporidium* species in apparently healthy and diarrheic human population using Modified Ziehl-Neelsen technique.**

Type of samples	No. of samples	Positive cases	
		Number	%
Individuals with GIT disturbances	103	15	14.56
Apparently healthy individuals	74	2	2.7
Total	177	17	9.60

**Table (3): Prevalence of *Cryptosporidium* spp. in human population according age and sex using Modified Ziehl-Nelseen technique.**

Sex	Female		Males		Total	
	Number	+ve cases	Number	+ve cases	Number	+ve cases
Age						
1-10 years	43	8 (18.6%)	52	5 (9.61%)	95	13 (13.68%)
11-60 years	34	3 (8.82%)	48	1 (2.08%)	82	4 (4.87%)
Total	77	11 (14.28%)	100	6 (6%)	177	17 (9.60%)

**Table (4): Comparison of Modified Ziehl-Neelsen technique and AC ELISA test for detection of *Cryptosporidium* oocyst in feces of human and calves.**

Type of samples	No. of samples	MZN technique	ELISA test
Diarrheic humans	79	9 (11.39%)	10 (12.65%)
Diarrheic calves	10	1 (10%)	1 (10%)
Total	89	10 (11.23%)	11 (12.35%)

at a rate of 12%, 2.7%, 6.97% and 0% respectively. All positive calves and lambs were suffered from diarrhea (some of them had watery diarrhea). Calves usually become infected with *Cryptosporidium* between one and four weeks of age, and the duration of infection is short, lasting around two weeks, (Fayer et al., 1998b and Handley et al., 1999). *Cryptosporidium* spp. were identified in the feces of livestock (either normal or diarrheic) in Egypt and all-over the world by many authors as, Wahba (1994) 5.3% in lambs and 2.5% in goats at Qalubia Governorate, Abdel-Wahed (1999) 68.30% in lambs aged less than one month, Khalil (2000) 16.5% in sheep and 29.1% in goats, Rhee, et al. (1991) "22% in calves", Wee, et al. (1996) "14.4% in calves", Learmonth, et al. (2003) "0.27% in cattle and 4.55% in calves", Mahdi and Ali (2002) "20% in cattle, 13.3% in sheep and 12% in goats", Chalmers et al. (2002) "43% in healthy sheep" and Monica et al., (2004) "35.5% out of 971 calves". The infection by *Cryptosporidium* spp. is probably present in every domestic cattle herd in the world with asymptomatic infections. Prolonged oocyst excretion by adult cattle recognized as a major and continuous source of environmental contamination. Accordingly, they constitute potential sources for newborn calves contract the infection at a very young age (Tzipori and Ward, 2002). The negative result in sheep in this study may be due to the low number of examined sheep or the possible limited level of *Cryptosporidium* infection in the examined area. This finding indicated that calves constituted a major risk as a source of cryptosporidiosis in the environment, consequently persons handling or in contact to calves are at greater risk of zoonotic infection from *Cryptosporidium* (Monica, et al., 2004).

The overall positive rate of cryptosporidiosis among the 177 individuals examined was 9.60 % of which 14.56% out of 103 were suffered from diarrhea and 2.7% out of 74 were apparently healthy (Table 2). *Cryptosporidium* is a highly infectious protozoan that causes diarrheal illness around the world. The human host range is broad and includes immunocompromised people with AIDS, children in developing countries and immunocompetent individuals. Within many of these groups, the manifestations of disease are diverse, ranging from asymptomatic infections to life-threatening illness (Douglas, 1999). In a review of over 130,000 presumably immunocompetent patients with diarrhea, 6.1% in

developing areas and 2.1% in developed areas with diarrhea had *Cryptosporidium* infections (Adal et al., 1995). Among the HIV-positive patients with diarrhea, the percentages of *Cryptosporidium* infections rose to 24% and 14% (Adal et al., 1995). Also Cho, et al. (1993) reported 22% fecal oocyst positive rate out of 230 outpatients of private hospital. The low level of *Cryptosporidium* infection (2.6%) reported in apparently healthy individuals indicated that infection are rare in persons without symptoms, suggesting that *Cryptosporidium* should not be regarded as an opportunistic parasite (Navin, 1985). On the other hand, excretion of oocysts of *Cryptosporidium* by persons not complaining from any specific clinical symptoms such as diarrhea indicated that some individuals may remain asymptomatic during infection and therefore there was a carrier status of cryptosporidiosis (Checkley et al., 1997). There is circumstantial evidence of human cryptosporidiosis associated with exposure to infected livestock, particularly young cattle, animal manure and contaminated water (Fayer et al., 2000 and Thompson, 2003). Although farm workers and visitors to farms might have contracted cryptosporidiosis by direct contact, indirect zoonotic transmission of *Cryptosporidium* of cattle origin via water has long been considered to be the most important zoonotic source of human infection (Casmir, et al., 1997).

The positive rate of cryptosporidiosis in the examined females (16.66%) was higher than that of males (7.5%) (Table, 3). Children in the age group 1-10 years showed higher infection rate (13.68%) than that in the age group ranged from 11-60 years (6.66%). This result was similar to that reported by Jae et al. (2004) who detected cryptosporidiosis in females and males at a rate of 4.8% and 2.6% respectively. In developing countries, cryptosporidiosis is a common cause of persistent diarrhea among children (Leav, 2003). *Cryptosporidium* oocysts could be detected in children by Laubach, et al. (2004) at a rate ranged from 16-44%. They found that female children in 2 to 5 year age group were having a higher prevalence of infection than males. Out of 532 children with persistent diarrhea, 166 (31.2%) were found to harbor *C. parvum* (Tumwine et al., 2003). Casemore (1990), the peak incidence of cryptosporidiosis was in children aged 1-5 years. But Chai, et al. (2001) the peak positive rates in older age groups. On the other hand Xiao, et al. (2001)

found no significant difference in infection due to age. Children had a tendency to be less aware of hygiene practices and played in dirty yards contaminated with fecal material, so the probability to be get infected is high.

For comparison of Modified Ziehl-Neelsen technique and ELISA test for detection of *Cryptosporidium* oocyst in stool, 79 samples from diarrheic persons and 10 samples from diarrheic calves were subjected to analysis. The results obtained (Table 4) indicated that there was no significant difference between the 2 tests, although ELISA test was found to give higher positive rates (12.55%) than Modified Ziehl-Neelsen technique (11.39%). Previously, the "gold standard" for detecting *Cryptosporidium* oocysts in stool was a modified acid-fast stain of a fecal smear. However, the sensitivity for detection of *Cryptosporidium* is substantially better with enzyme-linked immunosorbent assay (Katanik *et al.*, 2001).

The stool immunoassays provide adequate sensitivities, cost-effective and rapid results, particularly in a potential waterborne outbreak situation and in patients with low parasitic numbers (DuPont *et al.*, 1995 and Fayer *et al.*, 1998a). In patients who are infected with *Cryptosporidium*, the use of routine diagnostic method such as modified acid-fast staining may be insufficient to demonstrate the presence of this organism (Current and Garcia, 1991 and Kehl *et al.*, 1995). As acute infections with *Cryptosporidium* resolve, the number of oocysts dramatically decreases. Also, the number of *Cryptosporidium* oocysts passed by patients, including those with human immunodeficiency virus disease, varies from day to day and week to week. None of these techniques allows discrimination regarding the species of origin of the oocysts. Only genetic techniques, including PCR, can help determine the possible source and risk to human health. During this study we found that most samples of humans and animals had a mixed infection of *Cryptosporidium* spp. As the sizes of the oocysts in feces were not even. Future study will be necessary to verify the mixed infection status in both animals and humans.

### References

- Abdel-Wahed, M. M. (1999): *Cryptosporidium* infection among sheep in Qalubia Governorate, Egypt. *J. Egypt. Soc. Parasitol.*, 29 (1): 113-118.
- Adal, K. A.; Sterling, C. R. and Guerrant, R. L. (1995): *Cryptosporidium* and related species. In *Infections of the gastrointestinal tract*. Blaser, M. J.; Smith, P. D.; Ravdin, J. I.; Greenberg, H. B. and Gurrant R. L., Ravin Press, New York (ed.), 1107-1128.
- Angus, K. W. (1983): *Cryptosporidiosis in man, domestic animals and birds: a review*. *J.R. Soc. Med.*, 76: 62-70.
- Blanshard, C.; Jackson, A. M.; Shanson, D. C.; Francis, N. and Gazzard, B. G. (1992): *Cryptosporidiosis in HIV seropositive patients*. *Q. J. Med.*, 85: 813-823.
- Casemore, D. P. (1990): Epidemiological aspects of human cryptosporidiosis. *Epid. Infect.*, 104: 1-28.
- Casmir, E. T.; Fayer, R. and Ronald, S. (1997): *Cryptosporidiosis-human and animal epidemiology*. In *Cryptosporidium and cryptosporidiosis* Boca Raton Fla (Fayer, R., ed.). CRC Press, 65-92.
- Chai, J. Y.; Kim, N. Y. and Guk, S. M. (2001): High prevalence and seasonality of cryptosporidiosis in small rural village occupied predominantly by aged people in the republic of Korea. *Am. J. Trop. Med. Hyg.*, 56: 518-522.
- Chalmers, R. M.; Elwin, K.; Reilly, W. J.; Irvine, H.; Thomas, A. L. and Hunter, P. R. (2002): *Cryptosporidium* in farmed animals: the detection of a novel isolate in sheep. *Int. J. Parasitol.*, 32 (1): 21-5.
- Checkley, W.; Gilman, R. H. and Epstein, L. D. (1997): Asymptomatic and symptomatic cryptosporidiosis: their acute effect on weight gain in Peruvian children. *Am. J. Epidemiol.*, 145: 156-163.
- Cho, M. H.; Kim, A. K. and Im, K. (1993): Detection of *Cryptosporidium* oocysts from outpatients of the Severance hospital. *Korean J. Parasitol.*, 31: 193-199.
- Crawford, F. G. and Vermund, S. H. (1988): Human cryptosporidiosis. *Crit. Rev. Microbiol.*, 16: 113-159.
- Current, W. L.; Reese, N. C.; Ernest, J. V.; Bailey, W. S.; Heyman, M. B. and Weinstein, M. D. (1983): Human cryptosporidiosis in immunocompetent and immunodeficient persons: studies of an outbreak and experimental transmission. *N. Engl. J. Med.*, 308: 1252-1257.
- Current, W. L. and Garcia, L. S. (1991): *Cryptosporidiosis*. *Clin. Microbiol. Rev.*, 3: 325-358.
- DuPont, H. L.; Chappell, C. L.; Sterling, C. R.; Okhuysen, P. C.; Rose, J. B. and Jakubowski, W. (1995): The infectivity of *Cryptosporidium parvum* in healthy volunteers. *N. Engl. J. Med.*, 332: 855-859.
- Douglas, P. C. (1999): New lights into human cryptosporidiosis. *Microbiol. Reviews*, 12 (4): 554-563.
- Fayer, R.; Trout, J. M. and Jenkins, M. C. (1998a): Infectivity of *Cryptosporidium parvum* oocysts stored in water at environmental temperatures. *J. Parasitol.*, 84: 1165-1169.
- Fayer, R. and Ungar, B. L. P. (1986): *Cryptosporidium* spp and *Cryptosporidiosis*. *Microbiol. Rev.*, 50: 458-483.
- Fayer, R.; Gasbarre, L.; Paquali, P.; Canals, A.; Almeria, S. and Zrlenga, D. (1998b): *Cryptosporidium parvum* in bovine neonates: dynamic, clinical, parasitic and immunologic patterns. *Int. J. Parasitol.*, 28 (1): 49-56.
- Fayer, R.; Morgan, U. and Steve, J. (2000): Epidemiology of *Cryptosporidium*: transmission, detection and identification. *Int. J. Parasitol.*, 30: 1305-1322.
- Handley, R. M.; Cockwill, C.; McAllister, T. A.; Jelinski, M.; Morck, D. W.; Olson, M. E. (1999): Duration of naturally giardiasis and cryptosporidiosis in dairy calves and their association with diarrhea. *J. Am. Vet. Med. Assoc.*, 214: 391-396.
- Henriksen, S. A. and Pohlenz, J. F. L. (1981): Staining of *Cryptosporidium* by a modified Ziehl-Neelsen technique. *Act. Vet. Scand.*, 22: 594-596.
- Jae, R. Y. U.; Jung-Kyu, L.; Min, S.; Seok, I. I. K.;

- Woon-Mok, S.; Sun, H.; Hae-Yeon, C. and Tong-Soo, K. (2004): Prevalence of Cryptosporidiosis among the villagers and domestic animals in several rural areas of Korea. *The Korean J. Parasitol.*, 42 (1): 1-6.
- Janoff, E. N. and Reller, L. B. (1987): Cryptosporidium species, a protean protozoan. *J. Clin. Microbiol.*, 25: 967-975.
- Katenik, M. L.; Schneider, S. K. and Rosenblatt, J. I. (2001): Giardia/Cryptosporidium rapid assay and ProSpecT Giardia/Cryptosporidium micplate assay for detection of Giardia and Cryptosporidium in fecal specimens. *J. Clin. Microbiol.*, 39: 4523-4525.
- Kehl, K. C.; Cicciello, H. and Havens, P. L. (1995): Comparison of four different methods for the detection of Cryptosporidium species. *J. Clin. Microbiol.*, 33: 416-418.
- Keusch, G. T.; Hamer, D.; Joe, A.; Kelley, M.; Griffiths, J.; and Ward, H. (1995): "Cryptosporidia - who is at risk?" *Schweiz. Med. Wochenschr.*, 125 (18): 899-908.
- Khalil, F. A. (2000): Studies on cryptosporidium in sheep and goats. Ph. D. Thesis. Fac. Vet. Med. Cairo Univ.
- Laubach, H. E.; Bentley, C. Z.; Ginter, E. L.; Spalter, J. S. and Jensen, L. A. (2004): A study of risk factors associated with the prevalence of *Cryptosporidium* in villages around Lake Atitlan, Guatemala. *Braz. J. Infect. Dis.*, 8: 4.
- Learmonth, J. J.; Ionas, G.; Pita, A. B. and Cowie R. S. (2003): Identification and genetic characterization of Giardia and Cryptosporidium strains in humans and dairy cattle in the Waikato Region of New Zealand. *Water Sci. Technol.*, 47 (3): 21-6.
- Leav, B. A.; Mackay, M. and Ward, H. D. (2003): Cryptosporidium species: new insights and old challenges. *Clin. Infect. Dis.*, 36: 903-908.
- Levine, D. N. (1984): Taxonomy and review of the coccidian genus *Cryptosporidium* (Protozoa, Apicomplexa). *J. Protozool.*, 31: 94-98.
- Mahdi, N. K. and Ali, N. H. (2002): Cryptosporidiosis among animal handlers and their livestock in Basrah, Iraq. *East. Afr. Med. J.*, 79 (10): 550-3.
- Meisel, J. L.; Perera, D. R.; Meligro, C. and Rubin, C. E. (1976): Overwhelming watery diarrhea associated with a *Cryptosporidium* in an immunosuppressed patient. *Gastroenterol.*, 70: 1156-1160.
- Meinhardt, P. L.; Casemore, D. P. and Miller, K. B. (1996): Epidemiologic aspects of human cryptosporidiosis and the role of waterborne transmission. *Epidem. Rev.*, 18: 118-136.
- Monica, S.; James, M. T.; Lihua, X.; Ling, Z.; Ellis, G. and Ronald, F. (2004): Prevalence and age-related variation of *Cryptosporidium* species and genotype in dairy calves. *Vet. Parasitol.*, 122 (2-21): 103-117.
- Navin, T. R. (1985): Cryptosporidiosis in humans: Review of recent epidemiologic studies. *Europ. J. Epid. (Historical Archive) Issue*, 1 (2): 77-83.
- Nime, F. A.; Burek, J. D.; Page, D. L.; Holscher, M. A. and Yardley, J. H. (1976): Acute enterocolitis in a human being infected with the protozoan *Cryptosporidium*. *Gastroenterol.*, 70: 592-8.
- Pohlenz, J.; Moon, H.W.; Cheville, N.F. and Bemrick, W.J. (1978): Cryptosporidiosis as a probable factor in neonatal diarrhea of calves. *J. Am. Vet. Med. Assoc.* 172.
- Rhee, J. K.; Seu, Y. S. and Park, B. K. (1991): Isolation and identification of *Cryptosporidium* from various animals in Korea. I. Prevalence of *Cryptosporidium* in various animals. *Korean J. Parasitol.*, 29: 139-148.
- Thompson, R. C. A. (2003): Molecular epidemiology of Giardia and Cryptosporidium infections. *J. Parasitol.*, 89: 134-140.
- Tumwine, J. K.; Kekitiinwa, A.; Nabukeera, N.; Akiyoshi, D. E.; Rich, S. M.; Widmer, G.; Feng, X. and Tzipori, S. (2003): *Cryptosporidium parvum* in children with diarrhea in Mulago Hospital, Kampala, Uganda. *Am. J. Trop. Med. Hyg.*, 68 (6): 710-5.
- Tzipori, S. and H. Ward (2002): Cryptosporidiosis: biology, pathogenesis and disease. *Microbs. and Infect.*, 4: 1047-1058.
- Tzipori, S.; Angus, K. W. and Gray, E. W. (1980a): Campbell, E.W. Gray. *Cryptosporidium*: evidence for a single species genus. *Infect. Immun.*, 30: 884-886.
- Tzipori, S.; Angus, K. W. and Gray, E. W. (1980b): Campbell, Vomiting and diarrhea associated with cryptosporidial infection. *N. Engl. J. Med.*, 303: 818.
- Wahba, A. M. A. S. (1994): Microbiological studies on Cryptosporidia and Sarcocystis in animals and their impact on their health. Ph. D. Thesis Parasitol. Fac. Vet. Med. Zagazig Univ.
- Weber, R.; Bryan, R.; Bishop, S.; Wahiquist, J.; Sullivan, D. and Juranek, D. (1991): Threshold of detection of *Cryptosporidium* oocysts in human stools: evidence of low sensitivity of current diagnostic methods. *J. Clin. Microbiol.*, 29:1323-1327.
- Wee, S.; Joo, H. D. and Kang, Y. B. (1996): Evaluation for detection of *Cryptosporidium* oocysts in diarrheal feces of calves. *Korean J. Parasitol.*, 34: 121-126.
- Xiao, L.; Bern, C. and Limor, J. (2001): Identification of 5 types of cryptosporidium parasites in children in Lima, Peru. *J. Infect. Dis.*, 183: 492-7.