EPIDEMIOLOGY OF CRYPTOSPORIDIUM SP UPSTREAM THE WATER TREATMENT PLANTS IN KPONG AND WEIJA, GHANA

G. T. MENSAH*, K. M. BOSOMPEM, P. F.AYEH-KUMI, J. OPPONG AND S. NIAMPOMA
(G.T.M. & S.N.: CSIR-Water Research Institute, P. O. Box AH38 Achimota; K.M.B.: Noguchi Memorial Institute for Medical Research, College of Health Sciences University of Ghana, Legon, P.F.A: Department of Microbiology, University of Ghana Medical and Dental School, College of Health Sciences, University of Ghana, Korle-Bu, J.O.: Department of Animal Biology and Conservation Science, University of Ghana Legon).
**Corresponding author's email: mensgato@yahoo.com*

Abstract

Cryptosporidium sp was first reported in 1907 in a wide range of domestic animals. It was later found to be zoonotic. These parasites of the Apicomplexa family are found in association with diarrhoea in calves and are water-borne. The organism is second to rotavirus as a causative agent of diarrhoea in new-born calves and infants. In order to estimate the human health risk in cattle rearing areas around water treatment plants, we measured the prevalence of *Cryptosporidium* oocysts in the faecal matter from four cattle kraals upstream in Joma near the Densu Dam at Weija in the Ga South Municipality and Kpong in the Lower Manya District of Southern Ghana. The Modified Ziehl-Neelsen (MZN) staining technique for *Cryptosporidium* oocysts was used. Of the 320 faecal samples for each species screened, 63 (19.7%) were positive for *Cryptosporidium*. Prevalence was higher in calves younger than three months of age, as compared to weaned calves and adults. Oocysts were detected in both diarrhoea and non-diarrhoea samples, with a significantly higher prevalence ($p < 0.05$) of oocysts shedding in diarrhoeic samples.

Introduction

Cryptosporidium sp was first reported in 1907 in a wide range of domestic animals and was later found to be zoonotic (Tyzzer, 1907).The parasite's infections were associated with diarrhoea in calves (Panciera et al 1971 & Tzipori and Ward 2002). The organism is reported to be second to rotavirus as causative agent of diarrhoea in new-born calves and infants.

Currently, the organism is known to cause diarrhoea in a wide range of animals including humans (Fayer and Ungar, 1986 and Giangaspero et al 2009).

Their oocysts which are resistant to dehydration in the environment are released with the faecal matter of the host (Fayer and Ungar, 1986; Fayer, Speer and Dubey, 1997) and can cause infection on release into the environment.

Infection is acquired when one drinks water contaminated with infected faecal matter, person to person infections have also been observed among children in day-care centres (Casemore, 1990; Cordell and Addis, 1994). One can also get infected through sexual practices which involve oro-anal contact as well as contact with items contaminated by faeces of infected calves and lamb (Casemore, 1990). Furthermore, indirect person to person or zoonotic transmissions occur through contaminated water used for recreation such as in swimming pools) as well as raw food in the form of meat, milk and farm made fruit juices (Casemore, Wright and Coop, 1997).

It has been reported that the normal procedure for water treatment cannot remove *Cryptospori-dium* from water, because a filter pore size of one micron or less is required to remove it. Also the oocysts are resistant to chlorine (Fayer, 2004).

In humans, cryptosporidiosis manifests in children less than five years of age (Anonymous, 2004), immuno-compromised adults such as those infected with HIV, those who have undergone organ transplants, daycare attendants and care givers to adult homes and HIV/AIDS patients (Karanis, Kourenti and Smith 2007).

The prevalence rate reported across the globe are: Africa 2.6 - 21.3%, Central and South America 3.2 - 31.5%, Asian countries 1.3 - 13.1%, Europe 0.1 - 14.1% and North America 0.3 - 4.3%. The better the sanitation and cleaner drinking water the less the prevalence rate (Ungar, 1990 and Fayer, 2004).

This study was undertaken to look at one of the potential sources of contamination of water bodies (cattle). Therefore, cattle farms in the environment upstream of the water treatment plant were investigated for the presence of the parasite which can be washed into the dam by surface run-off water during and after rainfall.

Experimental

Study Areas

The study was conducted in Weija in the Greater Accra Region and Kpong in the Eastern Region. The Kpong and Weija reservoirs supply drinking water to Accra the capital city of Ghana. Fig. 1 and Fig. 2 shows the selected communities for the study are upstream of the Weija and Kpong water treatment sites. Surface run-offs may carry contaminants from the surrounding communities' downstream serving as potential sources of faecal contamination to the Weija water body. The student's T-test and chi-square test were employed to test for the statistical analysis.

Fig. 1. A map of Lower Manya Krobo Municipality showing the study area.

Kpong is a town in the Manya Krobo Municipality of the Eastern Region of Ghana (Fig.1). The co-ordinates of Kpong are 6° 9' 0" North, 0° 0' 0" East. The Kpong dam was constructed in 1981 and is the second largest hydroelectric dam built in Ghana over the Volta River. It is downstream to the larger Akosombo dam and produces 148 to 160 MW of electricity. It was completed in 1982 and is managed by the Volta River Authority. The construction of the Akosombo dam in 1964 and the Kpong dam in 1981 on the Volta River created the Volta Lake in Ghana (Obeng-Asamoah *et al*. 1980). The impoundment of the river at Kpong caused an alteration of the existing ecological and biophysical processes in the river basin for both upstream and downstream and flooding of cultivated fields upstream. This has promoted the breeding of certain vectors of diseases, resulting in an increase in the populations of these vectors. Over the years, this has led to an increase in the prevalence of protozoan and other parasitic diseases in areas around the Volta Lake (Biney, 1987). In addition to this, there is increased disease transmissions associated with inadequate sanitary measures in the lakeside settlements (Gordon & Amatekpor, 1999). Since most people near the banks engage in open defecation, insanitary habits are prevalent leading to the spread of diseases in these communities which in turn to affect the socio-economic status of the people.

The main economic activities in Kpong are farming and fishing. The farmers use water from the Volta River to irrigate their farms which also serve as the main source of water for domestic chores (Obeng-Asamoah *et al*. 1980). The Migration of people from various districts to Kpong triggered the breakdown of cultural and social values leading to prostitution and an increased crime rate, increased prevalence of sexually transmitted diseases to the extent of becoming a common phenomenon and widespread migration of the populations, particularly of the young and energetic men and women (Gordon & Amatekpor, 1999). Curbing such activity which leads to the spread of

diseases, will help in alleviating poverty and will also increase life expectancy. The farms from where the faecal samples were collected are about 0.8 – 1km away from the upstream of the Kpong Lake..

Fig. 2. Map of Ga South Municipality showing Weija Dam (Study area)

Weija is a suburb of Accra and its geographical coordinates are 5° 34' 0" North, 0° 20' 0" West (Fig. 2). The Weija Lake, located 17 km west of Accra, capital of Ghana, has a shoreline of 48 km. According to Ampofo (1997), the creation of the Weija Lake in 1979 by the Government of Ghana to supply piped water and to support irrigation and fisheries programmes has provided ideal conditions for disease transmission. The Weija Reservoir has a surface area of about 300 ha and a depth of about 7m. The reservoir lies at 5^0 35^1 N and 0^0 22^1 W and has a mean annual inflow of 54.2 m^3 S^{-1}. Its catchment lies in the Coastal Savanna Zone where rainfall is seasonal with two peaks in June and September. The main economic activities in the catchment of both reservoirs are fishing and crop farming.

A survey of land-based sources of water pollution was undertaken in the catchment area of Weija Lake. There is an input of 12.42 $Mgyr^{-1}$ from settlements, 9.072 $Mgyr^{-1}$ from fertilizer input through runoff and the Densu River also has an input of 1,217.6 $Mgyr^{-1}$ into the Weija Lake (Biney, 1987). The sources of pollution from settlements include the dumping of solid and liquid wastes upstream of the Weija Lake and run-off from animal faeces and fertilizer inputs. The high mean nitrogen load of 3,003 Mgyr-1 can also be due to organic nitrogen input from domestic and agricultural origin. Joma is a small community located about 1 km away from the upstream of the Weija Lake (Biney, 1987). There are about three cattle ranches in the community and the cattle are released on free range each day. Samples of cattle faeces were taken from this community.

With a low income, advent of any disease will make economic life very difficult for these rural folks and therefore this study seeks to establish the presence of the parasite in the area and to establish the prevalence rates.

Collection of faecal samples

Study Population of cattle

Animals used in this study included cattle from four selected privately owned farms. Two of the farms were from Joma named as farms A and B and two from Kpong named as farms C and D. In all, 320 faecal samples from cattle were collected. Of these, 160 were sampled from Joma comprising of 80 each from farm A and farm B and 160 from the Kpong community comprising of 80 each from farm C and farm D. Samples were taken from all age groups. The animals were categorized as calves (< 3 months old), weaned (3 - 8 months old) and adults (> 8 months old). The animals live in open houses with muddy floors. All the animals graze on same pasture types therefore, having the same feed composition. The presence or absence of diarrhoea in the animals was also recorded.

Faecal samples

Fresh faecal samples of each animal within the farm were taken just after it had been dropped early in the morning and stored in a sterile plastic container. Faecal sampling was done twice on each farm on the same animal; the containers were labelled according to sites of collection.

Faecal samples were transported to the Noguchi Memorial Institute for Medical Research, preserved in 2.5% potassium dichromate at 4°C for laboratory analysis. Other information recorded included health status (diarrhoeic or non-diarrhoeic) and age of each animal. A total of 320 cattle were sampled from these farms from November 2008 to May 2009. Of the total number of study subjects 80 cattle were sampled from each of the four farms, faecal samples were taken from age groups which ranged from one month and 2 years. Of these, adults and weaned calves represented 33.4% each of the cattle population and 33.1% represented the calves.

Sample analysis

The Modified Ziehl-Neelson staining technique was used to prepare the faecal samples for examination under the light microscope to identify *Cryptosporidium* sp.

Modified Ziehl-Neelson technique

Faecal smears prepared on glass slides were air-dried and fixed for 5 minutes with methanol. The fixed smears were stained for 15 minutes with strong carbol-fuchsin, rinsed in running tap water and differentiated in acid alcohol (1% concentrated HCl in absolute alcohol) for 10 - 15 seconds. The smears were then rinsed again with tap water and counterstained with 0.4% malachite green stain for 30 seconds. The stained slides were then air-dried and examined under light microscope at x400 magnification (Casemore *et al.,* 1995).

Results

Prevalence of Cryptosporidium sp

Of the 320 faecal specimens screened in this study 63 (19.7%) were positive for *Cryptosporidium* oocysts. The highest prevalence of *Cryptosporidium* sp oocysts was on farm D in Kpong and the lowest prevalence was on farm A (Weija). Cattle from Kpong recorded a higher prevalence (23.8%) of *Cryptosporidium* sp. than in Weija (15.6%).

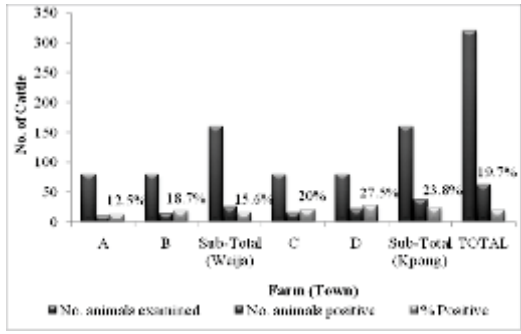

Fig. 3. Presenting the prevalence of Cryptosporidium oocysts in the screened farms.

Results on prevalence of infection among the age groups of cattle are given in Fig. 3. There was no definite difference in infections among the different age groups. *Cryptosporidium* sp. was most prevalent among the calves (28.3%) with the prevalence declining with increasing age. Cattle in the weaners group had a significantly higher prevalence of *Cryptosporidium* sp. than the adults ($p = 0.01$).

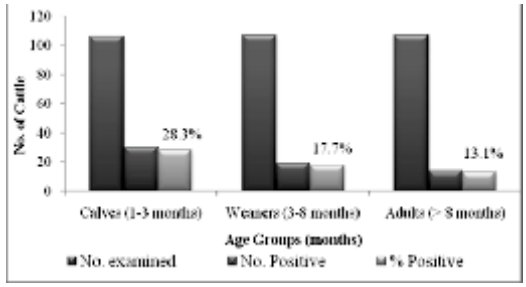

Fig. 4. Presenting the prevalence of Cryptosporidium oocysts in different age groups of cattle

Of the 320 samples examined, 68 had diarrhoea (watery stool), 33 of which were shedding *Cryptosporidium* sp. oocysts. 35 of the 68 diarrhoea cattle were calves, 57.6% of which were positive for *Cryptosporidium* sp. oocysts (Fig. 4).

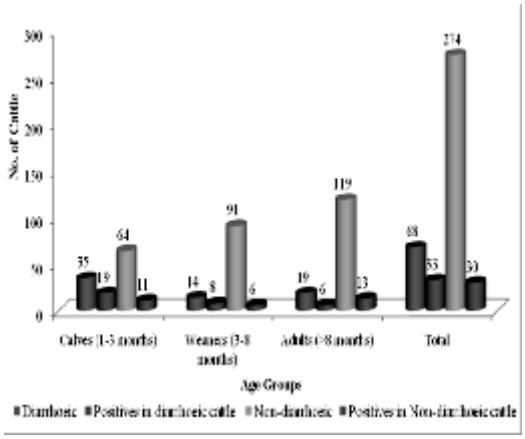

Fig. 5. Presenting association between the age of the cattle, presence of diarrhoea and occurrence of Cryptosporidium oocysts

Multiple Parasitic Infections

11 cattle (3.4%) were observed to be positive for both *Cryptosporidium* and *Giardia* during the study. Farm D (Kpong) recorded the highest prevalence of mixed infection (5%) and farms A and B (Weija) recorded the lowest (2.5%) (Fig. 5).

In addition, *Fasciola* spp. was identified in five of the 277 cattle that were negative for *Giardia* and four of the 254 cattle that were negative for *Cryptosporidium*. *Fasciola* spp. was also present in eight of the 17 cattle positive for *Giardia* only and 17 of the 66 *Cryptosporidium* positive cattle only. *Fasciola* spp. was found in all the cattle that were positive for both *Cryptosporidium* and *Giardia* parasites. In all 45 (14%) of the cattle were infected with *Fasciola* sp.

Furthermore, of the 320 samples examined 42 (13.1%) were also infected with different species of bacteria. 34 (80.9%) of these were identified in cattle that were negative for *Giardia* and eight in *Cryptosporidium* negative cattle.

Intensity of infection

Of the 63 *Cryptosporidium*-infected cattle, 31.7% had low counts (< 10) of parasites, 42.9% had moderate counts (10 – 20) and 25.4% had heavy counts of more than 20. Of the 17 cattle infected with *Giardia*, 52.9% registered less than 10 cysts, 29.4% had between 10 and 20 cysts and 17.6% had more than 20 cysts. Most calves recorded heavy counts and this was shown to decrease with increasing age as the adults recorded the lowest counts.

Discussion

Cryptosporidium sp are spread by natural host which include cattle, in the cattle they cause diarrhoea (Giangasper, Berrilli & Brandonisio, 2007) and this most at times affect the calves because their immune system is not well formed (Fayer, 2004). The cattle in this study were found to be infected with *Cryptosporidium* sp as reported by Giangasper and his team. The calves were found to have more of the parasites due to weak immune system and will therefore shed the parasites in their faecal matter into the environment and by that they contaminate water bodies through surface run-off water during and after it rains then they multiply in marshy areas as well as the surface water (Fayer, Speer & Dubey, 1997). *Cryptosporidium* sp. was most prevalent among the calves (28.3%) with the prevalence declining with increasing age. From the results obtained, the parasites were found in large numbers in the calves and the weaned calves than in the adults, 33 of which were shedding *Cryptosporidium* sp. oocysts. 35 of the 68 cattle with diarrhoea were calves, 57.6% of which were positive for *Cryptosporidium* sp. oocysts.

Conclusions

In this study, *Cryptosporidium infections* have been observed to be common in cattle in the study area *(*19.7*%)*. Prevalence was found to be high in calves less than 3 months old and declined with age. There was no observed significant difference in the seasonal transmission of the parasite though there was a high prevalence rate in the wet season than in the dry season ($p = 0.01$).

Also, the zoonotic potential of cattle in the transmission of *Cryptosporidium* infections in the communities cannot be ruled out as 10 oocysts are enough to cause infections. The farmers and their families interact with animals daily and dropped faecal matter on their compounds is not cleaned often; their food and water can be contaminated with the parasites increasing the infection rates.

Recommendations

1. In this study no significant difference was observed between seasonal transmissions of the two diseases. It is recommended that further studies covering over 24 months be conducted to confirm this finding.
2. This study has shown the presence of *Cryptosporidium* in cattle. It is recommended that further studies should employ the use of molecular methods to demonstrate that the parasites found in water and in cattle are the same species or assemblage that infects humans.
3. Studies by Mtambo (1995) in Tanzania showed that *Cryptosporidium* do infect wild animals who serve as a source of infection to domestic animals. There is no document on such finding in Ghana; further studies should therefore include wild animals to establish their zoonotic potentials.

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References

AMPOFO J. A. (1997) A survey of microbial pollution of rural domestic water supply in Ghana. *Int. J. Envir. Hlth Res*. **7**: 121 – 130.

BINEY, C. A. (1987) Changes in the chemistry of a tropical man-made lake, the Densu reservoir, during five years of impoundment. *Journal of Tropical Ecology*; **28**: 222 – 231.

CASEMORE, D (1990) Epidemiological aspects of human cryptosporidiosis. *Epidemiol. Infect*. **104**: 1 - 28.

CASEMORE, D. P., ARMSTRONG, M. & SANDS, R. L. (1995) Laboratory diagnosis of cryptosporidiosis and Giardiasis. *Journal of Clinical Pathology*; **38**: 1337 – 1341.

CORDELL, R. L. & ADDISS, D. G. (1994) Cryptosporidiosis in child care settings: a review of the literature and recommendations for prevention and control. *Pediat. Infect. Disease J.*, **13**: 310 – 317.

DÍAZ, V., CAMPOS, M., LOZANO, J., MANÃS, I. & GONZÁLEZ, K. (1996) Aspects of Giardiasis in Granada province (Southern Spain). *Veterinary Parasitology*; **64**: 171 – 176.

FAYER, R., SPEER, C. A. & DUBEY, J. P. (1997) The general biology of *Cryptosporidium*. *In Cryptosporidium and Cryptosporidiosis*, pp. 1–42. Edited by R. Fayer. Boca.

FAYER R. (2004) *Cryptosporidium*: a waterborne zoonotic parasite. *Veterinary Parasitology*; **126**:37 – 56.

FAYER R. & UNGAR, B. L. P. (1986) Cryptosporidium spp and Cryptosporidiosis. *Microbiol.* Rev.,**50**: 458 – 483.

GIANGASPERO, A., CIRILLO, R., LACASELLA, V., LONIGRO, A., MARANJI, M., CAVALLO, P., BERRILLI, F., CAVE, D.D., & BRANDONISIO, O. (2009) *Giardia* and Cryptosporidium in flowing water and harvested shellfish in a lagoon in Southern Italy. *Parasitol Int* **58**: 12 – 17.

GORDON, C. & AMATEKPOR, J. K. (1999) The Sustainable Integrated Development of the Volta Basin in Ghana, *Volta Basin Research Project, Accra*, 159p.

IQBAL, J., HIRA, R., AL-ALI, F. & PHILIP, R. (2001) Cryptosporidiosis in Kuwaiti children: seasonality and endemicity. *Clinical Microbiology and Infection*; **7**: 261 – 266.

JAKUBOWSKI, W. (1995) *Giardia* and Cryptosporidium: The Details. *Safe*

Drinking Water Act Seminar, U.S. Environmental Protection Agency.

KARANIS, P., KOURENTI, C. & SMITH, H. (2007) Waterborne transmission of protozoan parasites: a worldwide review of outbreaks and lessons learnt. *J. Water Health,* **5** (1):1 - 38.

KLAUS, R., RALF, I., THOMAS, W., SEIDU-KORKOR, A., ANYIDOHO, L., SAAD, E., AMOO-SAKYI, F., DANIKUU, F., OTCHWEMAH, R. N., SCHREIER, E., BIENZLE, U., KLAUS, S. & MOCKENHAUPT, F. (2007) Acute childhood diarrhoea in northern Ghana: epidemiological, clinical and microbiological characteristics. *BMC Infectious Diseases;* **7:**104.

LEACH, C. T., KOO, F. C., KUHLS, T. L., HILSENBECK, S. G. & JENSON, H. B. (2000) Prevalence of *Cryptosporidium parvum* infection in children along the Texas–Mexico border and associated risk factors. *American Journal of Tropical Medicine and Hygiene*; **62**: 656 – 661.

MTAMBO, M. M. A. (1995) The prevalence of *Cryptosporidium spp.* in cattle and wildlife inTanzania. *International Journal for Parasitology*; **28**: 111 - 120.

OBENG-ASAMOAH, E. K., JOHN, D. M. & APPLER, H. N. (1980) Periphyton in the Volta Lake; Seasonal changes on the trunks of flooded trees. *Hydrobiologia*; **76** (3): 191 – 200.

PANCIERA, R. J., THOMASSEN, R. W. & GARNER, F. M. (1971) Cryptosporidiosis in a calf. *Veterinary Pathology*; **8**: 479 - 484.

TZIPORI, S. & WARD, H. (2002) Cryptosporidiosis: biology, pathogenesis and disease. *Microbes and Infection*; **4**: 1047 – 1058.

TYZZER, E. E. (1907) A sporozoan found in the peptic glands of the common mouse. *Proceedings of the Society for Experimental Biology and Medicine* **5**, 12 - 13.

UNGAR, B. L. P. (1990) Cryptosporidiosis in humans (*Homo sapiens*). In: Dubey, J. P0., Speer, C. A. & Fayer, R., eds. *Cryptosporidiosis of man and animals*. Boca Raton, FL, CRC Press: 59 - 82.

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