CRYPTOSPORIDIOSIS IN A COMMERCIAL DAIRY CATTLE FARM IN MALAYSIA

NORHAMIZAH A.H., JULAIDA S., SLAMAH B., SAUDAH S. AND RASIDAH A.L.

Regional Veterinary Laboratory Johor Bahru, Lot PTB 11098, Jalan Taruka, Off Jalan Datin Halimah, 80350 Johor Bahru, Johor.

Corresponding author: norhamizah79@gmail.com

ABSTRACT. *Cryptosporidium* spp. was detected in 3 cows from rectal pinch samples. Direct smear stained with Acid Fast and Kinyoun stain was used to detect the organism. Subsequent samplings also indicated positive for Cryptosporidiosis, whereby one of the animals died due to dehydration and severe clinical signs of diarrhea. The farm had contaminated water supply where two out of the four ponds were positive for *Cryptosporidium* spp. whereas the municipal water supply was negative. The management of the farm was poor in terms of nutrition and cleanliness which led to *Cryptosporidium* spp. infection in the cattle compounded by stress factors. The mortality of the adult dairy cattle and calves was also high reaching up to 40%. The most common cause of death was leg weakness, severe dehydration and pneumonia in calves as a result of severe infections. Cryptosporidiosis is zoonotic and thus needs to be controlled to prevent outbreaks in the human population.

INTRODUCTION

Cryptosporidiosis is a disease which causes diarrhoea in the calves of dairy cattle. It results from infection by an obligate intracellular parasite namely *Cryptosporidium parvum* (OIE, 2005). The *C. parvum* lives in an oocyst which is found in manure excreted from infected calves. The oocysts of this parasite are circular in shape and the size is approximately 3 to 5 microns. It is normally found in calves 3 days to 3 weeks old (McCleese, 2002). Transmission of the oocysts usually occurrs by the fecal oral route. When a calf takes in water or food which is contaminated with manure from infected cattle, it comes into contact with the *cryptosporidium* eggs or oocysts. The ingested oocyst contains four sporozoites that are released through the oocyst membrane. These sporozoites multiply asexually in the intestine of the host. When mature, the sporozoite will form a thick-walled oocyst or a thin-walled oocyst (Juranek, 2000). The thick-walled oocyst is environmentally-resistant and passed in the faeces. The thin-walled oocyst can rupture and the sporozoites can infect new host enterocytes (cells that line the intestine) resulting in cell destruction which may lead to atrophy and fusion of intestinal villi (Kneen *et al*., 2000). As a result, the calf cannot absorb nutrients,

water, and essential electrolytes from the feed it eats. The calf becomes lethargic and weak and will show loose to watery stool that may be mild or severe in intensity (Hodge, 2004). The specimens for primary diagnosis should be collected during acute infection, and should be processed as soon as possible. Ideally, transportation systems should be selected to ensure that specimens arrive at the laboratory within 24 hours (OIE, 2008). Diagnosis is established microscopically, with the acid-fast Ziehl-Neelsen methods using unconcentrated or concentrated faecal smears (OIE, 2008). The procedure for Kinyoun staining is similar to the Ziehl-Neelsen but does not involve heating the slides being stained (Margaret *et al*., 2000). Both techniques will show *Cryptosporidium* spp. oocyst stained red in color, measuring about 3-5 µm.

The oocysts are resistant to chlorinated drinking water and difficult to filter as they are small in size. Cryptosporidiosis can infect humans as well as most other mammals. It is important to keep the pens clean and good personal hygiene should be practiced in order to prevent calves from getting infected with cryptosporidiosis.

MATERIALS AND METHOD

A private cattle farm complained of high mortality and severe diarrhoea in its animals. The investigation into the case involved collection of samples for diagnosis such as faecal, blood and water. One of the diseases suspected was cryptosporidiosis based on the clinical signs.

For this study three rectal pinch samples were taken for the first sampling and two rectal pinch samples from the second sampling from animals with clinical signs of diarrhea. The rectal pinches were packed in individual leak-proof plastic bags and, immediately sent to the laboratory. Direct unconcentrated faecal smear technique was used in this study (OIE, 2008). Moderately thick smears are recommended for this procedure. If the smear is too thin or thick, oocysts will be missed. An acceptable thickness can be achieved when either the hands of your watch or the print on this page can just be read when viewed through the preparation (Casemore *et al*., 1985). Smears were stained with acid fast-Ziehl-Neelsen and Kinyoun methods and observed under light microscope. For the Ziehl-Neelsen staining technique, the faecal smear was air dried, and the smear was completely covered with strong filtered Carbol Fuchsin which was poured onto the smear. The slide was flamed until the steam appeared and allowed to stand for 5 minutes. Then, the slide was washed off with water several times. Following this, slides were decolourised with 3% acid alcohol for 2 minutes and washed off again with water. Then methylene blue was poured completely to cover the slide for 30 seconds as a counter stain. Lastly, the slide was washed off with water and air dried (Carter, 1978). For the Kinyoun method, the faecal smear was prepared and air dried. Commercial Kinyoun was used

for this study. Firstly, Carbol Fuschin was completely poured on the air dried faecal smear and allowed to stand for 5 minutes. Following this, the slide was decolourised with acid alcohol for 30 seconds. Lastly, methylene blue was completely poured to cover the slide and allowed to stand for 20-30 seconds. Immediately after each step, the slide was washed with water several times. With the Kinyoun acid-fast method, the oocysts of *Cryptosporidium* spp. may stain pink to red to deep purple. Some of the four sporozoites may be visible in the *Cryptosporidium* oocysts. The background will stain blue. The oocysts are rounded and measure 3-5 µm (Garcia, 2001).

Concentrating of *Cryptosporidium* spp. oocysts by (Formol/ether) centrifugal sedimentation for water samples.

Five water samples from different sources of water supply for the affected farm were collected in sterilized glass containers. The detection of *Cryptosporidium* oocysts involved, concentration of water

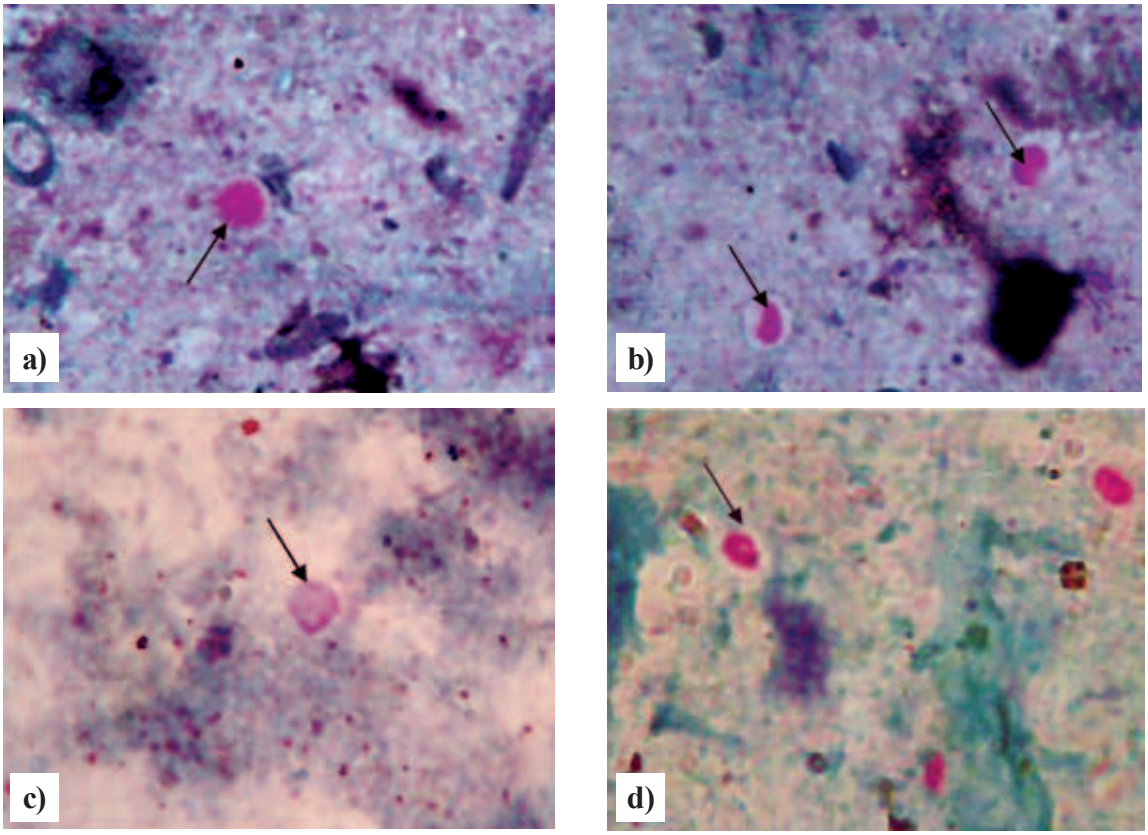

Figure 1. *Cryptosporidium* spp. under 1000x light microscope. (a) & (b) stained with Acid Fast Ziehl-Neelsen from faecal smear sample. (c) Stained with Acid Fast Ziehl-Neelsen from water sample. (d) Stained with Kinyoun from faecal smear sample.

sediments. The 1.5 litre water samples were individually centrifuged at 2500 rpm for 5 minutes. The supernatant was removed leaving 1 ml above the pellet. A small amount of 750 µl of the concentrated suspension of each water source was drawn out and formol/ether method of staining and identification was carried out (OIE, 2008). Smears were made on slides, air dried and were stained with acid fast-Ziehl-Neelsen and Kinyoun methods and observed under light microscope. The organisms appeared rounded or oval, pinkish red and measured 3-5 µm. Several oocysts were observed in each smear confirming the presence of the organism.

RESULTS

All three rectal pinch samples were positive for *Cryptosporidium* spp. and follow up samples 4 weeks after first sampling showed that two of the samples were also still positive. One of the affected cattle had also died due to severe dehydration. Results from the water supply showed that 2 ponds out of 4 ponds supplying water to the farm were positive for *Cryptosporidium* spp. where else the government water supply was negative for *Cryptosporidium* spp. Figure 1 shows *Cryptosporidium* spp. oocysts in faecal and water samples with the 2 stains.

DISCUSSION

Cryptosporidium parvum is one of several protozoal species that cause cryptosporidiosis, a parasitic disease of the mammalian intestinal tract and especially important as a zoonotic parasite.

In humans, primary symptoms of *C. parvum* infection are acute, watery, and non-bloody diarrhoea. *C. parvum* infection is of particular concern in immunocompromised patients, where diarrhea can reach 10–15L per day. Other symptoms may include anorexia, nausea/ vomiting and abdominal pain.

Infection is caused by ingestion of sporulated oocysts transmitted by the faecal-oral route. In healthy human hosts, the median infective dose is 132 oocysts. The general *C. parvum* life cycle is shared by other members of the genus. Invasion of the apical tip of ileal enterocytes by sporozoites and merozoites causes pathology seen in the disease.

Infection is generally self-limiting in immunocompetent people. In immunocompromised patients, such as those with AIDS or those undergoing immunosuppressive therapy, infection may not be self-limiting, leading to dehydration and, in severe cases, death.

The diagnosis of *C. parvum* consists of serological tests and microscopic evaluation of oocysts in stools using Kinyoun acid-fast staining. In most laboratories, the most convenient method of diagnosis is by Kinyoun's staining which is cheap and fast giving reliable results.

C. parvum is considered to be the most important waterborne pathogen in developed countries. It is resistant to all practical levels of chlorination, surviving

for 24 hrs at 1000 mg/L free chlorine. <http://www.dpd.cdc.gov/dpdx/HTML/Cryptosporidiosis.html>

Based on the results obtained, *Cryptosporidium* spp. was detected in 3 cows from rectal pinch samples using direct smear stained with Acid Fast and Kinyoun stain. The oocysts appear red after acid fast staining. All positive samples (100%) indicate that the dairy cattle were highly infected with Cryptosporidiosis. It has been reported recently that four species of *Cryptosporidium* mostly responsible for bovine cryptosporidiosis. However, of the four common *Cryptosporidium* spp. in cattle, *C. parvum* is the only recognised zoonotic (Leoni *et al.*, 2006).

In this study, all the three *Cryptosporidium*-positive dairy cattle had no obvious clinical signs at the time of sampling. Subsequent sampling that was carried out 4 weeks after first sample contained *Cryptosporidium*. However, one of the cattle died due to dehydration and severe clinical signs of diarrhea. Scouring cattle should be identified as early as possible since cattle can lose a great amount of fluid through its manure in a short period of time. This may result in dehydration and possibly death. If a calf is found to have very watery faeces, it should be isolated from the other calves immediately.

This study showed a higher prevalence when compared to prevalence studies by Nasir *et al.* (2009), Aiqin *et al.* (2009) and Santin *et al.* (2008). Nasir *et al.* (2009) found that out of a total of 500 faecal samples tested, 128 were positive showing an overall prevalence of *Cryptosporidium parvum* in dairy calves as 25.6%. Besides that, in a study done by Santin *et al.* (2009), 190 specimens were found to be *Cryptosporidium*-positive (19.2%) from a total of 990 specimens. According to Aiqin *et al.* (2009), 27 of the 507 faecal specimens from the six farms in Heilongjiang Province, China were diagnosed as *Cryptosporidium*-positive by microscopic examinations.

A cross-sectional study was conducted to determine prevalence and risk factors of cryptosporidiosis in bovine from two contrasting production system in and around Tanga municipality between May 2003 and January 2004. The study populations comprised 117 calves aged less than 3 months, randomly selected from 44 smallholders dairy and traditional managed herds, respectively. Individual calf and herd-level information was collected using a structured questionnaire and feacal samples were screened for *Cryptosporidium* spp. oocysts using the modified Ziehl-Neelsen method. Overall, 35% of the calves in the study were shedding *Cryptosporidium* spp oocysts, with at least one positive calf detected in 54.5% of herds. Independent risk factors for cryptosporidiosis were: age $\geq$1 to $\leq$2 months and level of cleanness of calf house floor categorized as dirty ($P < 0.05$). Similarly an increases risk of *Cryptosporidium* spp. infection was found in calves from smallholder dairy units compared to traditional herds ($P < 0.05$). The finding highlights that *Cryptosporidium* spp. is prevalent among calves in the

area under study. The high prevalence of cryptosporidiosis detected in this study suggests that it may have a significant impact on livestock industry and that the close interaction between cattle and human may play a role in zoonotic transmission to humans (Swai & Schoonman L, 2010).

Water sedimentation was used for the detection of *Cryptosporidium* oocysts in this study. It was found that 2 out of 4 ponds supplying water to the farm contains *Cryptosporidium* spp. whereas the source of water supplied by the municipal government does not contain *Cryptosporidium* spp. The results indicate that the management of farm was poor in terms of nutrition and cleanliness which led to *Cryptosporidium* spp. infection in the cattle compounded by stress factor. The mortality of the adult dairy cattle and calves was high as it was reaching up to 40%. Severe infection of *Cryptosporidiosis* may results in leg weakness, severe dehydration and pneumonia, which mainly causes the death of calves.

CONCLUSION

Cryptosporidium lives in the intestine of infected humans, birds, fish reptile and other animals. Millions of *Cryptosporidium* can be released in a single bowel movement from an infected specimen. Infections occur after oral consumption of the parasite. *Cryptosporidium* may be found in soil, food, water, or surfaces that have been contaminated with the faeces from infected humans or animals. The present study demonstrated that *Cryptosporidium* infection of cattle is important and hence further studies are needed to show its relative importance, mainly in the neonatal diarrhea syndrome.

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REFERENCES

1. Aiqin, L., Rongjun, W., Yihong, L., (2009). Prevalence and distribution of *Cryptosporidium* spp. in dairy cattle in Heilongjiang Province, China. Parasitol Res **105:** 797-802
2. Anonymous, (2004). "Cryptosporidiosis." [Internet] *Institute for International Cooperation in Animal Biologics*. Available from: http://www.cfsph.iastate.edu/FastFacts/pdfs/cryptosporidiosis_F.pdf [Accessed December 28, 2010]
3. Hodge, R, (2004). "Cryptosporidiosis in Calves." [Internet]. Available from: http://mildpdf.com/search-cryptosporidiosis-in-calves.html [Accessed December 28, 2010]
4. Juranek, D, (2000). "Cryptosporidiosis: Sources of Infection and Guidelines for Prevention." [Internet] *Center for Disease Control and Prevention (CDC).* Available from: http://www.cdc.gov/ncidod/dpd/parasites/cryptosporidiosis/crypto_sources_of_infect.htm. [Accessed December 28, 2010]
5. Kneen, B, Darling, S, Lemley, A. (2000) "Cryptosporidium: A Waterborne Pathogen". [Internet] USDA Water Quality Program, Cornell Cooperative Extension. Available from: http://www.cce.cornell.edu/factsheets/wq-factsheets/cryptosporidium.htm [Accessed December 28, 2010]
6. Leoni, F., Amar, C., Nichols, G., Pedraza-Dı´az, S., McLauchlin, J., (2006). Genetic analysis of Cryptosporidium from 2414 humans with diarrhoea in England between 1985 and 2000. J. Med. Microbiol. **55:** 703–707
7. McCleese, J, Phillips, D.A., Scharko, P., (2002). "Cryptosporidiosis in Dairy Calves." [Internet]. Available from: http://www.uky.edu/Ag/AnimalSciences/dairy/extension/nut00054.pdf [Accessed December 28, 2010]

8. Nasir, A., M. Avais, M.S. Khan and N. Ahmad, (2009). Prevalence of *Cryptosporidium parvum* infection in Lahore (Pakistan) and its association with diarrhea in dairy calves. *Int. J. Agric. Biol.,* **11:** 221–224
9. Santin, M., Trout, J.M., Fayer R., (2008). A Longitudinal study of Cryptosporidiosis in dairy catte from birth to 2 years of age. Veterinary Parasitology **155** (2008): 15-23.
10. OIE Terrestial Manual (2008). Chapter 2.9.4 Cryptosporidiosis. Available from:http://www.oie.int/eng/norms/mmanual/2008/pdf/2.09.04_CRYPTO.pdf
11. Casemore D.P., Armstrong M. & Sands R.L (1985). Laboratory diagnosis of cryptosporidiosis. J.clin.pathol., **38:** 1337-1341.
12. Magaret A. Bartelt, (2000), F.A. Davis Company. "Diagnostic Bacteriology: A Study Guide". Available from: http;//en.wikipedia.org/wiki/Kinyoun_stain"
13. "Cryptosporidiosis." Laboratory Identification of Parasites of Public Health Concern. CDC. 5 Sept 2007. <http://www.dpd.cdc.gov/dpdx/HTML/Cryptosporidiosis.html>
14. Swai E.S. and Schoonman L. (2010) Investigation into the Prevalence of Cryptosporidium Infection in Calves among Small-Holder Dairy and Traditional Herds in Tanzania. Vet Med Int. **22:** 2010:676451.
15. Garcia, L.S. 2001. *Diagnostic Medical Parasitology*, ed. 4., ASM Press, Washington, D.C., p. 723.
16. Carter, G.R. 1978. Diagnostic Procedures in Veterinary Microbiology. ed. 2,. Charles C. Thomas Pub. Springfield, Illinois, p. 299.