

Infectious Causes of Neonatal Diarrhea in Cattle in Kuwait with Special Reference to Cryptosporidiosis

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Abstract: During September to November, 2007, an outbreak of neonatal calf diarrhea occurred in Friesian calves on 8 dairy farms at Sulaibiya area, Kuwait. From each farm, fecal and blood samples were collected randomly from diarrheic calves aged 1-3 weeks and sent to the veterinary laboratories, public authority of agriculture affairs and fish resources to be tested for the evidence of cryptosporidium, rotavirus, coronavirus and Bovine Viral Diarrhea (BVD) infection. Cryptosporidium oocysts were detected in 38.8% (31/80) of the fecal samples, rotavirus in 28.8% (19/66) and BVD virus antibodies 62% (49/79) in serum samples. Coronavirus was not detected in 66 fecal samples tested. Cryptosporidium was detected on the 8 farms tested and found concurrently with rotavirus on 6 farms. On one farm, cryptosporidium was the only detectable cause of diarrhea.

Key words: New born calf, cryptosporidium, rota, BVD, infection, Kuwait

INTRODUCTION

In Kuwait, neonatal diarrhea is a major problem in dairy farms, leading to high mortality and is hindering the sustainable development of the dairy sector. Enteropathogens including *E. coli* (K99), salmonella, klebsiella, rotavirus and cryptosporidium were reported to be the causative agents of diarrhea in pre-weaned dairy calves (Razzaque *et al.*, 2001, 2006).

Cryptosporidiosis is a protozoal disease that causes enteritis and diarrhea in many species of mammals including man (O'Donoghue, 1995). Worldwide, it has become a major economic concern for livestock producers and public health officials (Casemore *et al.*, 1997; Guerrant, 1997). Recently, the disease has been recorded in Kuwaiti children (Sulaiman *et al.*, 2005). Information on the incidence of cryptosporidium in livestock from Kuwait is limited and none has been published. This study documents the occurrence of cryptosporidium infection among livestock in Kuwait and as a cause of diarrhea in new born calves.

MATERIALS AND METHODS

Country description: Kuwait is a small predominantly desert country; it has a hot dry summer and a mild winter with low and infrequent rainfall. Average autumn (September to October) temperature and relative humidity are 30.8°C and 38.3%, respectively. During the Iraqi invasion in 1991, the animal resources were almost wiped out. After the liberation of Kuwait, the government and private sector worked hard to reestablish the animal industry mainly through importation and the 2007 census records 27,000 cattle. Friesian dairy cattle were kept in sedentary large scale farms in Sulaibiya area (about 20 km from Kuwait city).

Sources and collection of samples: The samples received at the veterinary laboratories, public authority of agriculture affairs and fish resources form the basis of this study and data presented here. The sources of samples were dairy farms in Sulaibiya area during an outbreak of newborn calf diarrhea which occurred on 8 dairy farms in the period extended from September to November, 2007.

Morbidity rates ranged from 20-60% and mortalities reached up to 40% among calves at 3 weeks of age. The calves were initially treated with antibiotics. When they did not respond to antibiotic treatment, practicing veterinarians sent samples for parasitological and virological examination. Fecal and blood samples were collected from neonatal calves aged 1-3 weeks with severe and persisted cases of diarrhea. The fecal samples were collected by stimulating the anal sphincter with a sterile swab. The feces were placed in sterile plastic cups with cover. Blood samples were collected in vials from the jugular vein. Samples were sent on ice to the laboratories by hand delivery within 2 h of collection. They were tested soon after submission.

A total 80 fecal samples were received at parasitology laboratory for detection of cryptosporidium. About 66 fecal samples were submitted to the virology laboratory for detection of rotavirus and coronavirus as well as 79 blood samples for detection of bovine virus diarrhea antibodies.

Parasitological examination: For detection of cryptosporidium oocysts smears were made from fecal samples and intestinal contents on glass slides using a sterile swab. The dried smears were stained by the modified Ziehl-Nielsen method (Garcia *et al.*, 1983) and were screened under oil immersion magnification (1000 X).

Virological examination: Commercial ELISA kits (BioX Diagnostics, Belgium) were used to test the presence of rota and corona viral antigens in fecal samples and intestinal contents and for the detection of BVD virus antibodies in sera. The ELISA tests were performed according to the manufacturer's instructions. In addition, the fecal samples were tested by immunochromatography for qualitative detection of rotavirus antigen (Antigen Animal Genetics, Inc, Korea).

RESULTS AND DISCUSSION

Different enteropathogens were detected on the 8 dairy farms and are shown in Table 1. Cryptosporidia were detected in animals from all the farms surveyed. Oocysts were found in 31 of 80 (38.8%) samples submitted. However, there were differences in the prevalence among farms. On farm 2, 4 of 5 calves examined were positive. Whereas on the farms 3 and 6, 2 out of 12 (16.7%) were infected.

One farm (farm 5) was not sampled for virological examination. Antibodies against BVD virus were detected on the other seven farms tested at an infection rate of 49/79 (62%) whereas rotavirus was found on 6 farms at an

Table 1: Prevalence of enteropathogens detected on eight farms at Sulaihiya area

Farms	Enteropathogens detected		
	Cryptosporidium No tested/positive	Rotavirus No tested/positive	BVDv No tested/positive
1	26/10	21/10	10/8
2	5/4	3/1	11/5
3	12/2	10/0	10/3
4	10/5	10/3	14/14
5	3/2	-	-
6	12/2	10/2	10/3
7	6/4	6/2	14/14
8	6/2	6/1	10/2
Total	80/31	66/19	79/49
Percentage	38.3	28.8	62.0

infection rate 19/66 (28.8%); animals on only one farm (farm 3) were negative for this virus. None of the samples was positive for coronavirus. This study documents the occurrence of cryptosporidium in several Kuwaiti farms. The results indicate that this protozoan is an important contributor to the etiology of diarrhea in calves. The study also reveals the widespread occurrence of cryptosporidium on dairy farms and this is in accordance with other studies in the Middle East (Brenner *et al.*, 1993; Arslan *et al.*, 2001). The overall prevalence of cryptosporidium infection in this study (38.8%) was higher than those reported in diarrheic calves in other countries, e.g., Israel (16.5%) (Brenner *et al.*, 1993), Trinidad and Tobago (9.4%) (Kaminjolo *et al.*, 1993), Korea (14.4%) (Sunghwan *et al.*, 1996) and Turkey (25.7%) (Arslan *et al.*, 2001) and lower than those reported in Spain (52.3%) by De la Fuente *et al.* (1999) and by Maddox-Hyttel *et al.* (2006) (96%) in Denmark.

Factors that may contribute to these differences between studies could be related to variations in age groups examined and the method employed for oocyst detection. Furthermore, it could be a reflection of differences in management systems. Kaminjolo *et al.* (1993) found that the dissemination of oocysts is favored in intensive and semi-intensive systems than in extensive system. Herd size could also influence the detection of cryptosporidium on a farm. Garber *et al.* (1994) demonstrated that large herds were significantly more likely to have cryptosporidium-infected calves than were small herds. Large herds may have a heavier pathogen load because of greater density of animals.

Only one age group of calves (between 1-3 weeks old) was tested in this study as this is the age group most commonly affected clinically (Naciri *et al.*, 1999; Santin *et al.*, 2004; Maddox-Hyttel *et al.*, 2006).

In this study, a modified Ziehl-Nielsen method was used to detect cryptosporidium oocysts in fecal smears. The acid fast stain is reliable and the most widely used for identification of cryptosporidium oocysts (Fayer and

Ungar, 1986). However, the species was not identified. Majewska *et al.* (2000) found that there was agreement among the results obtained by Ziehl-Nielsen, immunologic and molecular methods for detection of oocysts in the fecal samples. Although, morphological identification of oocysts or detection of antibodies by immunological methods provide generalized prevalence data for the presence of cryptosporidium however, neither method is sufficient to identify species and genotypes of cryptosporidium. Molecular methods are the best to validate the species and genotypes of the organism (Santin *et al.*, 2004).

Using PCR analysis, Sulaiman *et al.* (2005) identified *C. parvum* in the great majority of Kuwaiti cryptosporidium infected children (58/62). Cryptosporidium infection seems common in human populations (Shehab *et al.*, 2001; Iqbal *et al.*, 2001; Sulaiman *et al.*, 2005). The results from studies in the medical field and the highlight the importance of cryptosporidiosis as a zoonotic disease. Infected calves excrete large number (millions) of *C. parvum* oocysts (Naciri *et al.*, 1999). The oocysts excreted into the environment are very resistant and remain infective for several months either in soil or water threatening livestock and human populations. However, no epidemiological information was available to elucidate the sources of infection with *C. parvum* in human population. Using the effluent as manure and contact with domestic animals could be sources of infection to humans. Even in this study, no data were collected to identify the risk factors of transmission of cryptosporidium on dairy farms. Therefore, further investigations are needed.

In field outbreaks, diarrheal disease has a complex etiopathogenesis and cryptosporidium may occur concurrently with other pathogens including rotavirus, coronavirus, enterotoxigenic *E. coli* and salmonella (De la Fuente *et al.*, 1999).

On all farms (6/7) tested, there were mixed infections with cryptosporidium and rotavirus. This observation is consistent with other studies which have found both pathogens to be the most commonly detected agents in young calves with diarrhea (McDonough *et al.*, 1994; De la Fuente *et al.*, 1999).

On one farm, cryptosporidium was recorded as the only cause of calf diarrhea which supports the finding of De la Fuente *et al.* (1999) that this parasite is the primary cause of acute diarrhea in new born calves.

Detection of BVD virus antibodies was high (62%). The same observation was recorded by Harkness *et al.* (1978) who found 92.5% of cattle population in England

had BVD virus antibodies. BVD virus infected animals can shed large quantities of virus particles every day in their urine, feces and saliva which are considered a source of infection to other animals in the herd giving reason of high infection rates. BVD infection is usually inapparent to mild disease of short duration; occasionally it appears as an overt disease with clinical signs but mostly in adult cattle (Baker, 1995). Therefore in this study, BVD virus can be incriminated as one of the etiological agents of diarrhea in calves <3 weeks of age. However, BVD virus has the ability to cause immunosuppression which increases the susceptibility to infection and enhances the severity of calf diseases including neonatal calf diarrhea (Scott *et al.*, 1980; Grooms *et al.*, 2009).

Results of rotavirus detection were 19/66 (28.8 %); found on 6 farms. Animals in only one farm (farm 3) were negative for this virus. None of the samples was positive for coronavirus. Similar results were obtained by Selim *et al.* (1991) who examine faecal samples from calves under 1 year of age from three dairy farms and one village in selected areas of Bangladesh. The samples were tested by an Enzyme-linked Immunosorbent Assay (ELISA) to detect the presence of rotavirus antigen. Of the 402 calves tested, 49 (12.2%) were positive. Rotavirus was most commonly found in calves of 1 week of age or less (up to 22.2% in one group) but was not found in any calves over 6 months of age.

Rotavirus infections constitute one of the main causes of economic loss owing to growth delay, birth of weak calves and high mortality rates in herds. Many studies have showed that rotavirus was the predominant factor in 50% of newborn deaths due to diarrhea in calves (Garcia-Sanchez *et al.*, 1993; Yavru *et al.*, 2008).

Razzaque *et al.* (2006) studied prevalent calf diseases in Kuwait that may affect health performance and found that pneumonia and diarrhoea were the main causes of mortality in dairy calves. The main causes of diarrhea were infection by *E. coli* (24%), Rotavirus (28%), Salmonella (49%) and Cryptosporidium species (100%). In the current study, nearly similar results for rotavirus detection (28.8%) were obtained while cryptosporidium detection was significantly less than that found in their studies (38.8%).

Management and housing may also play an important role in the transmission of cryptosporidium on animal farms. In Kuwaiti farms, cattle are kept in pens with dirt flooring; accumulated manure is generally removed once a month. Calves are left with their dams for 3 days to allow them to drink colostrum and later they are fed milk replacer.

All these are factors that could facilitate the transmission of infection to newborns from soil, dams or farm workers.

In addition, resistant cryptosporidium oocysts that pass from the host into the environment are infective and need no time to mature. Therefore, they may not be affected much by harsh environmental conditions as they are transmitted directly to calves. This direct transfer could explain the widespread occurrence of cryptosporidium on animal farms in Kuwait where the weather is extremely hot and inhospitable to any pathogenic agents.

CONCLUSION

This study documents the occurrence of cryptosporidium in livestock in Kuwait and reveals the importance of this pathogen of as a cause of diarrhea among calves in Kuwait.

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