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PRELIMINARY STUDY ON CRYPTOSPORIDIOSIS IN LIVESTOCK FROM KUWAIT

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Abstract

Out of 225 fecal samples (40 of calves, 128 of lambs and 57 of goat kids) received in the Veterinary Laboratories, Kuwait, 43 (19.1%) were found to be positive for *Cryptosporidium* using the conventional method, modified acid-fast stain, and the immunologic technique, the immuno-chromatography assay. Calf fecal samples showed the highest infection rate (37.5%), followed by goat kids (21%) and then Lambs (12.5%). Results obtained by conventional method were found to be consistent with those detected using the immunologic technique.

Key words: Kuwait, Cryptosporidiosis, Livestock, Diagnosis microscopic, immunology.

Introduction

Infectious diarrhoea is common in children. Globally, it is the third leading cause of mortality in children less than 5 years, accounting for 800 000 deaths annually. In the Northern Territory, Australia, Indigenous children are disproportionately burdened, with higher rates of hospitalisation and longer duration of illness than non-Indigenous children[2]. Prolonged and recurrent episodes of diarrhoeal illness can result in environmental enteropathy syndrome, and complications including severe dehydration, acidosis and hypokalaemia are more common (McLeod *et al*, 2014). The parasitic causes of diarrhoea *Cryptosporidium parvum, Entamoeba histolytica, Giardia lamblia* and *Strongyloides stercoralis*, may be accompanied symptoms were fever, headache, anorexia, vomiting, malaise, and myalgia (Badawy *et al*, 2012). *Cryptosporidium* sparked great public health interest after the large human waterborne outbreak in Milwaukee in 1993 (Morgan *et al*, 2000) and rapidly became recognized as one of the most serious and difficult to control waterborne pathogens. Subsequent, the zoonotic potential of *Cryptosporidium* was studied in one of the most densely populated worldwide regarding livestock and people (Mosier and Oberst, 2000; Moon *et al*, 2013; helmy *et al*, 2013). Cryptosporidiosis in Kuwaiti children was reported (Sulaiman *et al*, 2005; Iqbal *et al,* 2011) as well as Majeed *et al.* (2011) reported cryptosporidiosis calves.

The study aimed to evaluate whether the microscopic examination of modified Ziehl-Nielsen stained fecal smears and/or the immuno-chromatogrphy rapid test offer an advantage for routine detection of *Cryptosporidium* in the livestock.

Materials and Methods

The current study focused on symptomatic farm animals from Kabd, Salmi, Abdeli and Wafra areas over the period from January-August 2013.

The materials were based on the fecal samples and data obtained from the Veterinary Laboratories as well as fecal samples obtained from the rectum of carcasses brought to the laboratory for P.M.

The collected samples were sealed in labeled plastic containers and sent to the veterinary laboratory, for diagnosis of gastrointestinal parasites. The individual data included the age, sex, number of animals in the herd and number of deaths.

A total, 13 cattle farms and 40 sheep and goat flocks were sampled where 40 bovine, 128 ovine, and 57 goat kids' fecal samples were collected. Cattle were housed in intensive system, where very large numbers of animals were raised on limited space of

land, which meant that animals from the same herd were in direct contact with each other. Sheep and goats, on the other hand, were kept in semi-extensive system, where animals have the freedom to roam outdoors for grazing with minimal contact with other animals in the herd.

For the detection of *Cryptosporidium* oocysts, smears from fecal samples were prepared on glass slides using sterile swabs, dried and stained by modified Ziehl-Nielsen (acid-fast) method (Garcia *et al*, 1983), and examined by oil immersion lens for oocysts.

Commercial Vetexpert Rapid BoviD-4 Ag test kit is a solid phase immunochromatographic assay for the rapid, qualitative detection of *Cryptosporidium* antigen, Rotavirus antigen, Corona virus antigen and *Escherichia coli* k99 antigen in calf feces. The kit was used to test the presence of *Cryptosporidium* antigen in the fecal samples. This kit is a rapid immuno-chromatography assay for the qualitative detection of *Cryptosporidium* antigen; the test was performed according to the manufacturer's instructions. *Cryptosporidium* sensitivity 98.2%, specificity 99%

Results

Table 1: *Cryptosporidium* in fecal samples of livestock, received in the Veterinary Laboratory

Animals	No. tested	No. positive	Positive percentage
Calves	40	15	37.5
Lambs	128	16	12.5
Goat kids	57	12	21
Total	225	43	19.1

Discussion

Cryptosporidium parvum is a globally distributed protozoan parasite that is found in both vertebrates and invertebrates (Lima *et al*, 2011). Infections are transmitted by the fecal–oral route, or through contaminated food or water, and several major waterborne outbreaks have occurred (Yoder and Beach, 2010). Cryptosporidiosis represents a major health problem as it is a frequent cause of diarrhea in both immunocompetent and immunodeficient individuals (Rossignol, 2010). The risk of developing a severe disease differs depending on the personal immune status. In immunocompetent humans, exposure usually results in a self-limited disease manifested by watery diarrhea with a duration of about 2 weeks (El-Hamshary *et al*, 2008) In contrast, in immunocompromised patients the parasite is of particular clinical significance, since it may cause a severe persistent disease that may be life-threatening (Abdou *et al*, 2013). Generally speaking, Budu-Amoako *et al*. (2011) Foodborne illness associated with Cryptosporidium and Giardia from livestock.

In the present study, the overall infection rate of *Cryptosporidium* was calculated to be 19.1 %. The highest rate of infection was in calves (37.5%), followed by goat kids (21%), and while the lowest rate of infection was in lambs (12.5 %). The higher rate of infection in calves could be explained by the fact that cattle are raised in an intensive system where there are large numbers of the animals crowded in minimal space of land, which means higher contact among the animals in the herd and this would necessarily be reflected in the higher rate of infection and may require higher medical attention. The higher ratio of contact facilitates the spread of contamination through the deposition of large numbers of oocysts to the environment, and subsequently the dissemination of those pathogens to other animals in the farm.

Oocysts were only detected in young diarrheic animals (less than 1 month old).
This finding agreed with Kaminjolo *et al*, (1993) and Garcia *et al*, (2000). Cryptosporidiosis in ruminant species is typically symptomatic in the young. Among cattle, calves are susceptible to infection shortly

after birth and remain so for several months (Maikai *et al,* 2011). Infection in dairy calves is most often detected (via fecal oocyst shedding) between 8 and 15 days of age, whereas infection in beef calves most often occurs between 1 and 2 months of age (Garber *et al*, 1994; Park *et al*, 2012). Infection in lambs and goat kids is more common in animals' less than one month old (García-Presedo *et al*, 2013). This result could be justified by the fact that veterinarians tend to collect samples from sick animals with diarrhea. Other solid fecal samples were negative and from animals suffering from other health problem e.g. off-food, loss of body weight, alopecia etc., rather than diarrhea.

The present study and that of Majeed *et al.* (2011) revealed that *Cryptosporidium* infection occurs throughout the year, except in summer when ambient temperatures may reach 50°C. In general the weather of Kuwait is hot and arid, which is inhospitable to any pathogenic agents; however, 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 the harsh environmental conditions as they are transmitted directly to susceptible animals, particularly in intensive systems and overcrowded farms.

The results obtained from microscopic examination of stained fecal smears and the immuno-chromatography rapid test results were compatible, indicating that both tests can be used in the laboratory for fecal examination. Although the immuno-chromatography test is faster and easier to employ, it is much more expensive. Therefore, it was suggested that immunology test be used only in case an infection is detected by the conventional screening method, especially if the suspected animal carries a low numbers of oocysts (Ramirez *et al*, 2004).

Of interest, Areeshi *et al.* (2007) stated that the cumulative experience from Saudi Arabia as well as Kuwait, Oman, Jordan and Iraq suggested that *Cryptosporidium* is an important cause of diarrhea in man and cattle, but with limitations of the available data concerning these countries. Amer e*t al*. (2013) reported that the common occurrence of non-*C. parvum* species and IId subtypes in pre-weaned calves is a distinct feature of cryptosporidiosis transmission in dairy cattle in Egypt. The finding of the same two dominant IIa and IId C. parvum subtypes recently found in humans in Egypt suggests calves can be potential reservoirs of zoonotic cryptosporidiosis. Adamu *et al*. (2014) in acrosssectional study of 520 HIV/AIDS patients found that *C. parvum* was a major cause of cryptosporidiosis in HIV-positive patients and zoonotic transmission was important in cryptosporidiosis epidemiology in Ethiopia. and confirmed that different *Cryptosporidium* species and subtypes are linked to different clinical manifestations.

Conclusion

The outcome results showed that the immuno-chromatography rapid test was compatable to the microscopic examination of stained smear. No doubt, in Kuwait cryptosporidiosis is one of main causes of diarrhea and livestock loss. Zoonotic transmission of *C. parvum* due to contact with calves is predominant among farm workers and their household members of this region and appropriate health measures must be applied to control the infection and decrease of zoonotic transmission of this parasite.

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