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Detection of faecal *Cryptosporidium parvum* antigen in diarrheic Holstein dairy cows

Badiei, K.*, Pourjafar, M. and Ghane, M.
Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran, Postal Code 71345, P.O. Box 1731, Shiraz, Iran
* Corresponding author e-mail: badiei33@gmail.com
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Abstract. Over a one-year period, based on a random cluster sampling design, 661 faecal samples from natural cases of diarrheic calves were taken in Fars province of Iran. The samples were taken from the 267 diarrheic calves of high and 394 diarrheic calves of average producing Holstein dairy cows. Faecal samples were collected directly from the rectum. Herd selection was based on geographical location and density of cattle in the region. Samples were collected based on 5 percent of herd population in 4 geographical regions: North, West, East and South of Fars province. The herds were stratified into small, medium and large size. Laboratory investigation consisted of a direct identification test for antigen of *Cryptosporidium parvum*. All herds had HPDC and APDC *Cryptosporidium*-infected diarrheic calves in their population. Diarrheic *Cryptosporidium* infected HPDC calves in southern region of Fars province were at much lower risk ($P<0.05$) than APDC calves. The rate of *Cryptosporidium* infection in diarrheic APDC calves in southern region of Fars province was highest when compared to other geographical locations. When considering the effect of age, diarrheic *Cryptosporidium* affected APDC Holstein calves of younger dams (>2 to 3years) showed a higher rate of infection when compared to diarrheic HPDC *Cryptosporidium* infected ones. There were no differences among the occurrence of *Cryptosporidium* infection in diarrheic HPDC and APDC calves of different herd size groups.

INTRODUCTION

Diarrhoea in newborn farm animals, particularly calves under 30 days of age is one of the most common disease complexes that the large-animal clinician encounters in practice (Smith, 2009). It is a significant cause of economic loss in cattle herds. The disease is affected by the intrinsic characteristics of the calf, its nutritional and immunological status, management of the herd, environment and various infectious agents (Bendali *et al*., 1999). Calves are at the greatest risk of developing diarrhoea in the first month of life and the incidence of diarrhoea decreases with age (Frank & Kaneene, 1992; Bendali *et al*., 1999). *Cryptosporidium* is a small protozoan parasite that infects the microvillous region of epithelial cells in the digestive and respiratory tract of vertebrates. It is an obligate intracellular parasite of man and other mammals, birds, reptiles and fish (Current & Blagburn, 1990). Cryptosporidiosis is an important and established cause of calfhood morbidity and sometimes high mortality rates among farm animals in bovines and has been implicated in outbreaks of diarrhoea in mammals (Björkman *et al*., 2003; Ulutaş & Voyvoda, 2004; Paul *et al*., 2009). Two species of *Cryptosporidium* have been identified in cattle: *Cryptosporidium parvum* (cattle genotype) in the intestine and *Cryptosporidium andersoni* in the abomasum (Sréter *et al*., 2000; Olson *et al*., 2004). *Cryptosporidium parvum* has been identified in 155 species of mammal including domestic animals. Preweaned

ruminants, especially calves, are especially vulnerable to infection (Nydam *et al.*, 2001; Fayer 2004). Calves usually become infected with *C. parvum* between 1 and 4 weeks of age (Ongreth & Stibbs, 1989; Uga *et al.*, 2000; Becher *et al.*, 2004). The cattle genotype of *C. parvum* also infects other mammals including humans. Past breeding strategies for dairy cattle have been very effective in producing rapid genetic gain to achieve industry targets and raise profitability (Zenger *et al.*, 2007). This type of selection may affect other systems such as immunity system. The difference in genetic potential and immunity (specific and non specific immunity) and other unknown factors in diarrheic calves of high and average producing Holstein dairy cows may affect the level of *Cryptosporidium* infection in diarrheic calves. In one instance, Kawakami *et al.* (2010) reported that calf diarrhoea in the early lactation period would be caused partly due to immaturity of leukocyte innate immunity. In view of this hypothesis, we decided to investigate the occurrence of *C. parvum* in faecal samples of diarrheic calves of high and average producing Holstein dairy cows while considering the geographical location, parity and herd size.

MATERIALS AND METHOD

Over a one-year period (from January to December 2009), based on a random cluster sampling design, 661 faecal samples from natural cases of diarrheic calves were taken by veterinary staff in Department of Large Animal Internal Medicine of Shiraz Veterinary School and veterinary practitioners in Fars province (Iran). The samples were taken from the 267 diarrheic calves of high (average 305-d milk production was approximately 7340 kg per cow) and 394 diarrheic calves of average producing (average 305-d milk production was approximately 3800 kg per cow) Holstein dairy cows. Faecal samples were collected directly from the rectum in sterile glass bottles, chilled and submitted for the laboratory diagnosis. Faecal samples were obtained in the first day of the onset of diarrhoea from non-treated calves up to 35 days of age. Faecal consistency was scored on a 4-point scale (Larson *et al.*, 1977). For this study, a score of 3 or 4 indicated the presence, and a score of 1 or 2 the absence of diarrhoea. The median age of the studied calves was 13 days and the age at which the calves were first fed colostrums was almost the same. Cows were never vaccinated against *C. parvum* infection. Herd selection was based on geographical location and density of cattle in the region. Samples were collected based on 5 percent of herd population in 4 geographical regions: North, West, East and South of Fars province (Iran). The herds were stratified into small (50–100 cows), medium (101–200 cows) and large size (>200 cows). Laboratory investigation consisted of a direct identification test for antigen of *C. parvum* (Bovine *Cryptosporidium* ELISA kit, BIO K 346 (Bio-X Diagnostics Sprl, Belgium).

Data were computed using Epi Info Version 6.04 (Dean *et al.*, 1994). The true prevalence was calculated using the following formula described by Rogan & Gladen (1978): True prevalence = (Apparent Prevalence + specificity -1) / (sensitivity + specificity -1). Statistical analyses for two way tables were tested using one-tailed Fisher's exact test with a value of 0.05.

RESULTS

The apparent and true prevalence of cryptosporidial infection in HPDC and APDC diarrheic calves are shown in Table 1. The rates, risk and odds ratio and results of one-tailed Fisher's exact probability test of *Cryptosporidium* infection in four different geographical locations (north, west, east, and west) of HPDC and APDC diarrheic calves are shown in Table 2. The odds ratio compared the relative odds of *Cryptosporidium* infection in diarrheric faeces of high and average producing dairy calves. An odds ratio lesser than 1 in *Cryptosporidium*-infected diarrheic calves indicated that the condition was more likely to occur in the APDC diarrheric calves. The risk ratio

compared the probability of *Cryptosporidium* infection in diarrheic calves of HPDC and APDC groups rather than the odds. All herds had HPDC and APDC *cryptosporidium*-infected diarrheic calves in their population. Diarrheic *cryptosporidium*-infected HPDC calves in southern region of Fars province were at much lower risk ($P<0.05$) than APDC calves. The rate of *cryptosporidium* infection in diarrheic APDC calves in southern region of Fars province was highest when compared to other geographical locations. The results of the infection rate in *Cryptosporidium*-infected diarrheic Holstein calves (HPDC and APDC) of different age groups of Holstein dams are shown in Table 3. When considering the effect of age, diarrheic *Cryptosporidium* affected APDC Holstein calves of younger dams (>2 to 3years) showed a higher rate of infection when compared to diarrheic HPDC *Cryptosporidium*-infected ones ($P<0.05$). There were no differences among the occurrence of *Cryptosporidium* infection in diarrheic HPDC and APDC calves of different herd size (Table 4).

DISCUSSION

Apparent prevalence, although useful as a consistent index, may underestimate the true prevalence of disease. The difference between true and apparent prevalence represents the accuracy of the diagnostic test used to assess the prevalence in the sample being tested. The low difference between the true and apparent prevalence of *Cryptosporidium* infection in diarrheic faecal samples of HPDC and APDC in our study represents the degree of accuracy of ELISA test for *Cryptosporidium* antigen detection in faecal samples used in this study. de la Fuente *et al*. (1998) stated that commercial ELISAs are being used increasingly to detect enterophathogens in faeces samples from calves and they have the advantage of not requiring special equipment or expertise and therefore, they are suitable for small laboratories.

Our study showed that the diarrheic APDC and HPDC calves in southern region, experienced more episodes of *Cryptosporidium* infection than other regions. There are three distinct climatic regions in the Fars Province of Iran. Firstly, the mountainous area of the north and northwest with moderate cold winters and mild summers. Secondly, the central regions, with relatively rainy mild winters, and hot dry summers. The third region located in the south and southeast, has moderate winters with very hot summers. de Graaf *et al*. (1999) found that seasonal variation in the prevalence of bovine *C. parvum* infection, may be owing to climate factors and various managemental factors such as seasonal production of livestock. It has been reported that the absorption of immunoglubulins (Ig) may be affected by the environment in which the calf is born (Quigley, 2007). Extreme cold (Olson *et al*., 1980a), but not moderate cold (Olson *et al*., 1981a,b), reduces the absorption of Ig by calves. The effects of ambient temperature outside the thermoneutral range for calves may involve direct effects on intestinal absorption and transport (Olson *et al*., 1981a) as well as the ability of the calf to stand and nurse (Olson *et al*., 1980b).

Most of the samples taken in southern region were obtained from those herds that had exactly lower quality management and sanitary practices. Although at first glance it seemed that the difference in the occurrence of *Cryptosporidium* may be related to the different climatic condition in Fars province, the management and sanitary practices also differed meaningfully in these areas. Björkman *et al*. (2003) reported that *C. parvum* was found in faeces from calves sampled during all seasons. An absence of association between season of the year and prevalence of the parasite was also reported by BWade *et al*. (2000). Therefore these results may be really related to lower quality management and sanitary practices rather than climatic conditions.

It also seems that either the heat stress or lower quality management and sanitary practices in southern region of Fars province

probably had markedly affected colostral composition or Ig content in diarrheic HPDC and APDC *Cryptosporidium* affected calves. It has been stated that cold, wet, windy weather during the winter months in temperate climates and hot humid weather during the summer months may be associated with an increased incidence of dairy calf mortality due to diarrhoea (Smith, 2009). Nardone *et al.* (1997) reported that colostrum yield was not reduced when Holstein heifers were exposed to high ambient temperature. However, total fat, lactose, energy, crude protein (CP), IgG, and IgA were lower than those for heifers maintained in a thermoneutral environment.

It was also noted that under the same conditions, diarrheic APDC calves in southern region of Fars province were at much higher risk than HPDC diarrheic calves to *Cryptosporidium* infection ($P<0.05$). It is probable that the level of stress experienced by these calves was higher than HPDC ones. These stress factors were unknown but may include those that adversely affect specific and innate immune defenses (nonspecific immunity). Stress is generally considered to suppress the immune system and may lead to an increase in the occurrence of disease in the presence of a pathogen. It has been stated that pituitary adrenocorticoptropin hormone (ACTH) travels through the blood to the adrenal cortex, where cells of the zona fasciculata secrete glucocorticoids (Fulford & Harbuz, 2005), with cortisol being the primary glucocorticoid in swine and cattle (Minton, 1994). Stress hormones released in response to activation of the hypothalamic-pituitary-adrenal (HPA) axis [corticotropin releasing factor (CRF), ACTH, and cortisol] have all been shown to have an effect on aspects of the immune system (Salak-Johnson & McGlone, 2007). It has been shown that incubation of cattle and porcine immune cells with cortisol suppresses lymphocyte proliferation, interleukin-2 (IL-2) production, and neutrophil function (Westley & Kelley, 1984; Blecha & Baker, 1986; Salak *et al.*, 1993).

The role of different genetic composition of HPDC and APDC *Cryptosporidium* affected diarrheic Holstein calves on the level of stress experienced in the same environment may be another probability for difference in immune system condition. The stress responsiveness of an animal has also been shown to be affected by genetics. Blecha *et al.* (1984) reported that Angus and Braham x Angus cattle responded immunologically differently to shipping stress. Angus calves had greater total IgG and IgM titers against pig red blood cells compared with Simmental calves (Engle *et al.*, 1999). Large White pigs had greater poststress ACTH levels after exposure to a novel environment than did Meishan pigs (Desautes *et al.*, 1999). Studies by Sutherland *et al.* (2005, 2006) showed numerous breed effects on various immune components. Another probability leading to higher stress in APDC diarrheic calves may be higher attention given to the HPDC calves in the same environmental conditions.

It has been stated that one of the factors that can influence the quality (particularly Ig content) of colostrums is parity of the dam (Kruse, 1970; Roy, 1990). This fact was also demonstrated in our study and the rate of *Cryptosporidium* infection in diarrheic Holstein calves was higher in cows between 2 and 3 years old. Furthermore it was shown that the APDC diarrheic calves suffered more from *Cryptosporidium* infection than HPDC diarrheic calves. This fact may also be related to the better quality of colostrum in dams of diarrheic HPDC calves and the better specific and nonspecific immunity in them and higher colostrum yield in HPDC dams. Our results revealed that there are differences of *Cryptosporidium* infection in diarrheic HPDC and APDC calves and the rate of cryptosporidial infection in diarrheic HPDC and APDC Holstein calves can be affected by geographical location and parity of the dam. The lower rate of *Cryptosporidium* infection in HPDC diarrheic Holstein calves found in this study could be due to the better specific and nonspecific immunity (possibly related to genetic composition) in diarrheic HPDC calves and higher colostrum yield in HPDC dams.

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