Global Veterinaria 6 (5): 441-446, 2011
ISSN 1992-6197

Fecal *E. coli* F5 (K99) Antigen in Diarrheic Calves of High and Average Producing Holstein Dairy Cows

Khalil Badiei, Mehrdad Pourjafar and Mohsen Ghane

Department of Clinical Sciences,
School of Veterinary Medicine, Shiraz University, Shiraz, Iran

Abstract: Over a one-year period, based on a random cluster sampling design, 661 fecal samples from natural cases of diarrheic calves were collected from Fars Province of Iran. The samples were taken from 267 diarrheic calves of high and 394 diarrheic calves of average producing Holstein dairy cows. Fecal 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 *E. coli* F5 (k99). All herds had High Producing Dairy Cows (HPDC) and Average Producing Dairy Cows (APDC) *E. coli* infected diarrheic calves in their population. Diarrheic *E. coli* infected HPDC calves in southern region of Fars Province were at lower risk of diarrhea than APDC calves (P<0.05). When considering the effect of age, diarrheic *E. coli* affected APDC Holstein calves of younger dams (>2 to 3years) at a higher rate of infection when compared to diarrheic HPDC *E. coli* infected ones (P<0.05). The proportion of infected *E. coli* diarrheic HPDC and APDC calves of younger dams (>2 to 3years) were higher than HPDC and APDC calves of older dams (3 and greater than 4 years). There was no difference among the occurrence of *E. coli* infection in diarrheic HPDC and APDC calves of different herd size groups.

Key words: *E. coli* (k99) · Diarrheic calves · High and average producing cows

INTRODUCTION

Calf diarrhea remains the leading cause of mortality in dairy calves and an important cause of morbidity and mortality in calves, particularly in those under 30 days of age [1]. The incidence of diarrhea in calves decreases with age [2]. Several enteropathogens were recovered from neonatal calf with diarrhea, their relative prevalence varies geographically but the most common prevalent infections in most areas are *Escherichia coli*, rotavirus and coronavirus, *C. Perfringens*, *Salmonella* spp. and *Cryptosporidium* spp. [3].

Among these organisms, enterotoxigenic *E. coli* (ETEC) is a bacterium that normally lives in the intestine and is the most common type of colibacillosis of young animals (primarily pigs and calves). It is also a significant cause of diarrhea among travelers and children in the developing world [4]. Colonization of the small intestine by ETEC is facilitated by surface factors known as pili, which mediate its adhesion to the epithelium [5]. Infected animals are the main reservoir for *E. coli* and their feces are the major source of environmental contamination with those bacteria [6]. The expression of K99 fimbriae (or F5 ETEC) accounts for nearly all cases of ETEC infection found in newborn calves [7]. ETEC produce several toxins, including the heat labile enterotoxin (LT), which disrupts electrolyte balance in the gut endothelium [8].

Past breeding strategies for dairy cattle have been very effective in producing rapid genetic gain to achieve industry targets and raise profitability [9]. This type of selection may affect other systems such as immunity system. Under selective pressure from infectious microorganisms, multicellular organisms have evolved immunological defense mechanisms, broadly categorized as innate or adaptive [10]. On the other hand, acquired immunity does occur independently of innate immunity and depends on many nonspecific (physiology, environment, etc.) and specific (e.g. genetic control) factors [11]. In one instance,

Corresponding Author: K. Badiei, Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran. Postal Code 71345, P.O. Box 1731. E-mails: badiei33@gmail.com & badiei@shirazu.ac.ir.

Kawakami *et al.* [12] reported that calf diarrhea in the early lactation period would be caused partly due to immaturity of leukocyte innate immunity. The difference in genetic potential and specific and non specific immunity (genetic immunity) and other unknown factors in diarrheic calves of high and average producing Holstein dairy cows may affect the level of *E. coli* fecal infection in diarrheic calves. In view of this hypothesis, we decided to investigate the occurrence of *E. coli* F5 (k99) in the feces of diarrheic calves of high and average producing Holstein dairy cows, while considering factors such as geographical location, parity of dams and herd size.

MATERIALS AND METHODS

Over one-year period (from January to December 2009), based on a random cluster sampling design, 661 fecal samples from diarrheic calves were taken by veterinary stuff in Department of Large Animal Internal Medicine of Shiraz Veterinary School and veterinary practitioners in Fars Province (Iran). Two hundreds and sixty seven samples were collected from diarrheic calves of high producing (average 305-d milk production was approximately 7340 kg per cow) and 394 samples were obtained from diarrheic calves of average producing (average 305-d milk production was approximately 3800 kg per cow) Holstein dairy cows. Fecal samples were collected directly from the rectum in sterile glass bottles, chilled and submitted for the laboratory diagnosis. Fecal samples were obtained in the first day of the onset of diarrhea from non-treated calves up to 35 days of age. Fecal consistency was scored on a 4-point scale [13]. For this study, a score of 3 or 4 indicated the presence and a score of 1 or 2 the absence of diarrhea. The median age of the studied calves was 13 days and the age at which the calves were first fed colostrum was almost the same. Cows were never vaccinated against rotavirus 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 the antigen of *E. coli* [(ELISA kit for antigenic diagnosis of *E. coli* F5 (k99) in cattle, BIO K 345 (Bio-X Diagnostics Sprl, Belgium)]. Data were computed using Epi Info Version 6.04. The true prevalence was calculated using the following formula described by Rogan and Gladen [14]: 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 *E. coli* infection in high and average producing dairy diarrheic calves are shown in Table 1. The rates, risk and odds ratio and results of one-tailed Fisher's exact probability test in *E. coli* infection of the four different geographical locations (north, west, east and west) of High Producing Dairy Cows (HPDC) and Average Producing Dairy Cows (APDC) diarrheic calves are shown in Table 2.

Table 1: Mean apparent and true prevalence of *E. coli* K99 fecal infection in HPDC (n=267) and APDC (n=394) diarrheic calves

Groups	Apparent prevalence	True prevalence
HPDC	0.086	0.094
APDC	0.113	0.124

HPDC: High producing dairy cows; APDC: Average producing dairy cows

Table 2: Epidemiologic parameters affected Holstein diarrheic calves infection by *E. coli* K99 in the four different geographical locations of Fars Province

Geographical location	N0. of calves	*E. coli* K99 positive calves	*E. coli* K99 Negative calves	Rate	Risk ratio	Odds ratio	Significance
North	HPDC (72)	n=30	n=42	0.417	0.87	0.77	0.26
	APDC (94)	n=45	n=49	0.479	(0.61-1.22)	(0.41-1.44)*	
South	HPDC (58)	n=30	n=28	0.517	0.78	0.54	0.05
	APDC (92)	n=61	n=31	0.660	(0.58-1.04)	(0.27-1.06)	
East	HPDC (69)	n=25	n=44	0.360	0.8	0.7	0.16
	APDC (105)	n=47	n=58	0.448	(0.55-1.18)	(0.37-1.3)	
West	HPDC (68)	n=25	n=43	0.368	0.78	0.66	0.13
	APDC (103)	n=48	n=55	0.466	(0.54-1.14)	(0.35-1.24)	

HPDC: High producing dairy cows; APDC: Average producing dairy cows; *95% confidence interval

Table 3: Epidemiological parameters of *E. coli* K99 infected HPDC and APDC diarrheic Holstein calves in dams of different ages

Years	N0. of calves	*E. coli* K99 positive calves	*E. coli* K99 Negative calves	Rate	Risk ratio	Odds ratio	Significance
>2	HPDC (n=96)	n=49	n=47	0.51	0.8	0.6	0.03
	APDC (n-142)	n=90	n=52	0.63	(0.63-1.01)	(0.35-1.01)*	
3	HPDC (n=82)	n=27	n=55	0.33	0.8	0.71	0.15
	APDC (n=125)	n=51	n=74	0.40	(0.55-1.17)	(0.39-1.27)	
≥4	HPDC (n=89)	n=33	n=56	0.37	0.77	0.63	0.07
	APDC (n=127)	n=61	n=66	0.48	(0.55-1.06)	(0.36-1.1)	

HPDC: High producing dairy cows; APDC: Average producing dairy cows; *95% confidence interval

Table 4: Epidemiological parameters of *E. coli* K99 infected HPDC and APDC diarrheic Holstein calves of different herd size

Herd size	N0. of calves	*E. coli* K99 positive calves	*E. coli* K99 Negative calves	Rate	Risk ratio	Odds ratio	Significance
Small	HPDC (81)	n=36	n=45	0.440	0.83	0.7	0.13
	APDC (141)	n=75	n=66	0.530	(0.62-1.11)	(0.4-1.21)*	
Medium	HPDC (88)	n=42	n=46	0.477	0.97	0.94	0.47
	APDC (116)	n=57	n=59	0.490	(0.72-1.29)	(0.54-1.64)	
Large	HPDC (98)	n=37	n=61	0.378	0.8	0.69	0.10
	APDC (137)	n=64	n=73	0.467	(0.59=1.1)	(0.4-1.17)	

HPDC: High producing dairy cows; APDC: Average producing dairy cows; *95% confidence interval

The odds ratio compared the relative odds of *E. coli* infection in diarrheas of HPDC and APDC calves. An odds ratio lesser than 1 in *E. coli* infected diarrheic calves indicated that the condition was more likely to occur in the APDC diarrheic calves. The risk ratio compared the probability of *E. coli* infection in diarrheic calves in HPDC and APDC groups rather than the odds. All herds had HPDC and APDC *E. coli* infected diarrheic calves in their population. Diarrheic *E. coli* infected HPDC calves in southern region of Fars Province were at lower risk of diarrhea than APDC calves (P<0.05). The results of the infection rate in *E. coli* 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, the proportion of infected *E. coli* diarrheic HPDC and APDC calves of younger dams (>2 to 3 years) were higher than HPDC and APDC calves of older dams (3 and greater than 4 years). There was no difference among the occurrence of E. coli infection in diarrheic HPDC and APDC calves of different herd size groups (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 *E. coli* infection in diarrheic fecal samples of HPDC and APDC calves in our study represented the degree of accuracy of ELISA test for *E. coli* antigen detection in fecal samples used in this study. De la Fuente *et al.* [15] stated that commercial ELISAs are being used increasingly to detect enterophathogens in feces samples from calves and they have the advantage of not requiring special equipment or an 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 *E. coli* 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 has moderate cold winters and mild summers. Secondly, the central regions have relatively rainy mild winters and hot dry summers. The third region located in the south and southeast, has moderate winters with very hot summers. In some parts of this latter area, the temperature occasionally rises above 40 degree centigrade in summers. The absorption of immunoglobulin (Ig) may be affected by the environment in which the calf is born [16]. Extreme cold [17], but not moderate cold [18, 19], 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 [18] as well as the ability of the calf to stand and nurse [20].

Several studies have reported that ETEC diarrhea and asymptomatic infections in man are most frequent during warm periods of the year [21, 22]. As most of fecal samples taken in this area were obtained during summer, it seems that heat stress in southern region of Fars Province probably had markedly affected colostral composition and Ig content in HPDC and APDC *E. coli* 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 diarrhea [6]. Nardone *et al.* [23] reported that colostrum yield was not reduced when Holstein heifers were exposed to high ambient temperature. However, total fat, lactose, energy, 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 *E. coli* infected APDC calves in southern region of Fars Province were at higher risk than HPDC calves ($P<0.05$). It is probable that the level of stress experienced by these calves was probably 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 (ACTH) hormone travels through the blood to the adrenal cortex, where cells of the zona fasciculata secrete glucocorticoids [24], with cortisol being the primary glucocorticoid in swine and cattle [25]. Stress hormones released in response to activation of the hypothalamic-pituitary-adrenal (HPA) axis [corticotrophin releasing factor (CRF), ACTH and cortisol have all been shown to have an effect on aspects of the immune system [26]. It has been shown that incubation of cattle and porcine immune cells with cortisol suppresses lymphocyte proliferation, IL-2 production and neutrophil function [27].

The role of different genetic composition of HPDC and APDC *E. coli* 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.* [28] reported that Angus and Braham x Angus cattle respond immunologically different to shipping stress. Angus calves had greater total IgG and IgM titers against pig red blood cells compared with Simmental calves [29]. Large White pigs had greater post stress ACTH levels after exposure to a novel environment than did Meishan pigs [30]. Recently, studies by Sutherland *et al.* [31, 32] showed numerous breed effects on various immune components. Another probability leading to higher stress in APDC diarrheic calves might 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 [33]. This fact was also demonstrated in our study and the rate of *E. coli* 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 *E. coli* infection than HPDC diarrheic calves. This fact may also be related to the better quality of colostrums in dams of HPDC calves and the better specific and nonspecific immunity in them and higher colostrums yield in HPDC dams. It can be concluded that the rate of *E. coli* infection in diarrheic HPDC and APDC Holstein calves may be affected by geographical location and parity of the dam. The lower rate of *E. coli* infection in HPDC diarrheic Holstein calves found in this study could be due to the better specific and nonspecific immunity in HPDC calves and higher colostrums yield in HPDC dams.

ACKNOWLEDGMENTS

We gratefully acknowledge the financial support we received from the Management and Planning Organization of Fars Province (Iran).

REFERENCES

1. Constable, P.D., 2004. Antimicrobial use in the treatment of calf diarrhea. J. Vet. Intern. Med., 18: 8-17.
2. Frank, N.A. and J.B. Kaneene, 1992. Management risk factors associated with calf diarrhoea in Michigan dairy herds. J. Dairy. Sci., 76: 1313-1323.
3. García, A., J.A. Ruiz-Santa-Quiteria, J.A. Orden, D. Cid, R. Sanz, M. Gómez-Bautista and F.R. De la Fuente, 2000. Rotavirus and concurrent infections with other enteropathogens in neonatal diarrheic dairy calves in Spain. Comp. Immunol. Microbiol. Infect. Dis., 23: 175-183.
4. Nicklasson, M., J. Klena, C. Rodas, A.L. Bourgeois, O. Torres, A.M. Svennerholm and A. Sjoling, 2010. Enterotoxigenic *Escherichia coli* Multilocus Sequence Types in Guatemala and Mexico. Emerg. Infect. Dis., 16: 143-146.

5. Duchet-Suchaux, M., 1988. Protective antigens against enterotoxigenic *Escherichia coli* O101: K99, F41 in the infant mouse diarrhea model. Infect. Immun., 56: 1364-1370.
6. Radostits, O.M., C.C. Gay, K.W. Hinchcliff and P.D. Constable, 2007. Veterinary Medicine. 10th Ed, W.B. Saunders, England, pp: 304-374.
7. Jay, C.M., S. Bhaskaran, K.S. Rathore and S.D. Waghela, 2004. Enterotoxigenic K99+ *Escherichia coli* attachment to host cell receptors inhibited by recombinant pili protein. Vet. Microbiol., 101: 153-160.
8. Amanda, L., M. Horstman and J. Kuehn, 2000. Enterotoxigenic *Escherichia coli* Secretes Active Heat-labile Enterotoxin via Outer Membrane Vesicles. J. Biol. Chem., 275: 12489-12496.
9. Zenger, K.R., M.S. Khatkar, J.A. Cavanagh, R.J. Hawken and H.W. Raadsma, 2007. Genome-wide genetic diversity of Holstein Friesian cattle reveals new insights into Australian and global population variability, including impact of selection. Anim. Genet., 38: 7-14.
10. Lazarus, R., D. Vercelli, L.J. Palmer, W.J. Klimecki, E.K. Silverman, B. Richter, A. Riva, M. Ramoni, F.D. Martinez, S.T. Weiss and D.J. Kwiatkowski, 2002. Single nucleotide polymorphisms in innate immunity genes: abundant variation and potential role in complex human disease. Immunol. Rev., 190: 9-25.
11. Muir, W.M. and S.E. Aggrey, 2003. Poultry genetics, breeding and biotechnology. Cabi Publication, Cromwell Press, Trowbridge, UK, pp: 293-294.
12. Kawakami, S., T. Yamada, N. Nakanishi, Y. Cai and H. Ishizaki, 2010. Leukocyte phagocytic activity with or without probiotics in Holstein calves. Res. J. Biol. Sci., 1: 13-16.
13. Larson, L.L., F.G. Owen, J.L. Albright, R.D. Appleman, R.C. Lamb and L.D. Muller, 1977. Guidelines toward more uniformity in measuring and reporting calf experimental data. J. Dairy. Sci., 60: 989-992.
14. Rogan, W.J. and J. Gladen, 1978. Estimating prevalence from the results of screening test. Am. J. Epidemiol., 107: 71-76.
15. De La Fuente, R., A. García, J.A. Ruiz-Santa-Quiteria, M. Luzón, D. Cid, S. García, J.A. Orden and M. Gómez-Bautista, 1998. Proportional morbidity rates of enteropathogens among diarrheic dairy calves in central Spain. Prev. Vet. Med., 36: 145-152.
16. Quigley, J., 2007. Passive Immunity in Newborn Calves. Adv. Dairy Tech., 19: 247-265.
17. Olson, D.P., C.J. Papasian and R.C. Ritter, 1980. The effects of cold stress on neonatal calves. 2. Absorption of colostrum immunoglobulins. Can J. Comp. Med., 44: 19-23.
18. Olson, D.P., R.C. Bull, L.F. Woodward and K.W. Kelley, 1981. Effects of maternal nutritional restriction and cold stress on young calves: absorption of colostral immunoglobulins. Am. J. Vet. Res., 42: 876-880.
19. Olson, D.P., L.F. Woodward and R.C. Bull, D.O. Everson, 1981. Immunoglobulin levels in serum and colostral whey of protein-metabolizable energy restricted beef cows. Res. Vet. Sci., 30: 49.
20. Olson, D.P., C.J. Papasian and R.C. Ritter, 1980. The effects of cold stress on neonatal calves. 1. Clinical condition and pathological lesions. Can. J. Comp. Med., 44: 11-18.
21. Rao, M.R., R. Abu-Elyazeed, S.J. Savarino, A.B. Naficy, T.F. Wierzba, I. Abdel-Messih, H. Shaheen, R.W. Frenck, A.M. Svennerholm and J.D. Clemens, 2003. High disease burden of diarrhea due to enterotoxigenic *Escherichia coli* among rural Egyptian infants and young children. J. Clin. Microbiol., 41: 4862-4864.
22. Steinsland, H., P. Valentiner-Branth, M. Perch, F. Dias, T.K. Fischer, P. Aaby, K. Molbak and H. Sommerfelt, 2002. Enterotoxigenic *Escherichia coli* infections and diarrhea in a cohort of young children in Guinea-Bissau. J. Infect. Dis., 186: 1740-1747.
23. Nardone, A., N. Lacetera, U. Bernabucci and B. Ronchi, 1997. Composition of colostrum from dairy heifers exposed to high air temperatures during late pregnancy and the early postpartum period. J. Dairy. Sci., 80: 838-844.
24. Fulford, A.J. and M.S. Harbuz, 2005. An introduction to the HPA. In Handbook of Stress and the Brain, Steckler, T., N.H. Kalin and J.M.H.M. Reul, Eds., Elsevier, Amsterdam, pp: 43-66.
25. Minton, J.E., 1994. Function of the hypothalamic-pituitary axis and the sympathetic nervous system in models of acute stress in domestic farm animals. J. Anim. Sci., 72: 1891-1898.
26. Salak, J.L. and J.J. McGlone, 2007. Making sense of apparently conflicting data: stress and immunity in swine and cattle. J. Anim. Sci., 85: E81-88.

27. Salak, J.L., J.J. McGlone and M. Lyte, 1993. Effects of in vitro adrenocorticotrophic hormone, cortisol and human recombinant interleukin-2 on porcine neutrophils migration and luminol-dependent chemiluminescence. Vet. Immunol. Immunopathol., 39: 327-337.
28. Blecha, F., S.L. Boyles and J.G. Ryley, 1984. Shipping suppresses lymphocyte blastogenic responses in Angus and Brahman x Angus feeder calves. J. Anim. Sci., 59: 576-583.
29. Engle, T.E., J.W. Spears, T.T. Brown and K.E. Lloyd, 1999. Effect of breed (Angus vs Simmental) on immune function and response to a disease challenge in stressed steers and preweaned calves. J. Anim. Sci., 77: 516-521.
30. Desautes, C., A. Sarrieau, J. Caritez and P. Mormede 1999. Behavior and pituitary-adrenal function in large White and Meishan pigs. Domest. Anim. Endocrinol., 16: 193-205.
31. Sutherland, M.A., S.R. Niekamp, S.L. Rodriguez-Zas and J.L. Salak-Johnson, 2006. Impacts of chronic stress and social status on various physiological and performance measures in pigs of different breeds. J. Anim. Sci., 84: 588-596.
32. Sutherland, M.A., S.L. Rodriguez-Zas, M. Ellis and J.L. Salak-Johnson 2005. Breed and age affect baseline immune traits, cortisol and performance in growing pigs. J. Anim. Sci., 83: 2087-2095.
33. Roy, J.H.B., 1990. The Calf. Vol. I. Management of Health. Butterworths, London, pp: 53-117.