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# Fecal Rotavirus Antigen in Diarrheic Calves of High and Average Producing Holstein Dairy Cows

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**Abstract:** 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 rotavirus infection in diarrheic calves. Over one-year period, based on a random cluster sampling design, 661 fecal samples from natural cases of diarrheic calves were taken in Fars province (Iran). The samples were taken from the 267 diarrheic calves of high (HPDCs) and 394 diarrheic calves of average producing Holstein dairy cows (APDCs). 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 antigens of rotavirus. Age and Parity of the dam was also recorded. Diarrheic rotavirus infected HPDC calves in southern region of Fars province were at much lower risk ($P<0.05$) than APDC calves. All herds had HPDC and APDC rotavirus infected diarrheic calves in their population. When considering the effect of age and parity of dams, the diarrheic rotavirus affected APDC calves of younger cows (>2 to 3years) showed a higher rate of infection when compared to diarrheic HPDC rotavirus affected Holstein calves. The rate of rotavirus infection in diarrheic APDC calves in southern region of Fars province was highest when compared to other geographical locations. There was a significant difference between the prevalence of rotavirus infection in diarrheic HPDC and APDC calves of Southern region ($P<0.05$). There were no differences among the occurrence of rotavirus infection in diarrheic HPDC and APDC calves of different herd size groups. Our results revealed that there are differences of rotavirus infection in diarrheic HPDC and APDC calves.

**Key words:** High and low producing dairy cows · Calf diarrhea · Feces · Rotavirus infection

## INTRODUCTION

Diarrhea 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 [1]. It is a significant cause of economic loss in cattle herds. The condition is a complex, multifactorial disease that is affected by the intrinsic characteristics of the calf, its nutritional, immunological status, management of the herd, environment and various infectious agents [2]. Calves are at the greatest risk of developing diarrhea in the first month of life and the incidence of diarrhea decreases with age [2, 3]. Viral diarrheas are the cause of high mortality among children and animals, including many mammalian and avian species [4, 5]. Rotavirus, a genus of double-stranded RNA virus in the family reoviridae**,** is the chief etiologic agent of viral gastroenteritis in infants and young children and in the young of a large variety of animal species [6, 7]. Bovine rotavirus is a major cause of calf diarrhea, usually occurring in calves at 1±3 weeks of age [8, 9]. 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 rotavirus infectivity in diarrheic calves. In one instance, Kawakami *et al.* [10] reported that calf diarrhea in the early lactation period would be caused partly due to immaturity of leukocyte innate immunity. In view of this hypothesis, the aim of the current study is to investigate the occurrence of rotavirus in fecal samples of diarrheic calves of high and average producing Holstein dairy cows while considering factors such as-geographical location, parity of dams and herd size.

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## MATERIAL AND METHODS

Over a one-year period (from January to December 2009), based on a random cluster sampling design, 661 fecal samples from natural cases of 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). The samples were taken from the 267 diarrheic calves of high (average 305-day milk production was approximately 7340 kg per cow) and 394 diarrheic calves of average producing (average 305-day 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 [11]. 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 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 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 antigens of rotavirus (Bovine rotavirus ELISA kit, BIO K 343 (Bio-X Diagnostics Sprl, Belgium). Parity of the dam was also recorded.

Data were computed using Epi Info Version 6.04. The true prevalence was calculated using the following formula described by Rogan and Gladen [12]: 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 rotavirus infection in HPDC and APDC diarrheic calves were shown in table 1. The rates, risk and odds ratio and results of one-tailed Fisher Exact probability test of rotavirus infection in four different geographical locations (north, west, east and west) of HPDC and APDC diarrheic calves were shown in table 2. The odds ratio compared the relative odds of rotavirus infection in diarrheas of high and average producing dairy calves. An odds ratio lesser than 1 in rotavirus infected diarrheic calves indicated that the condition was more likely to occur in the APDC diarrheic calves. The risk ratio compared the probability of rotavirus infection in diarrheic calves of HPDC and APDC groups rather than the odds. All herds had HPDC and APDC rotavirus infected diarrheic calves in their population. Diarrheic rotavirus infected HPDC calves in southern region of Fars province were at much lower risk ($P<0.05$) than APDC calves. The rate of rotavirus 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 rotavirus infected diarrheic Holstein calves (HPDC and APDC) of different age groups of Holstein dams were shown in Table 3. When considering the effect of age, diarrheic rotavirus affected APDC Holstein calves of younger dams (>2 to 3years) showed a higher rate of infection when compared to diarrheic HPDC rotavirus infected ones ($P<0.05$) (Table 3). There were no differences among the occurrence of rotavirus infection in diarrheic HPDC and APDC calves of different herd size groups (Table 4).

Table 1: Mean apparent and true prevalence of rotavirus fecal infection in HPDC (n=267) and APDC (n=394) diarrheic calves

| Groups | Apparent prevalence | True prevalence |
|---|---|---|
| HPDC | 0.286 | 0.291 |
| APDC | 0.326 | 0.332 |

HPDC: High producing dairy cows

APDC: Average producing dairy cows

Table 2: Epidemiologic parameters in four different geographical locations (north, west, east, and west of Fars province) of HPDC and APDC rotavirus affected Holstein diarrheic calves

| Geographical location | N0. of calves | Rotavirus positive calves | Rotavirus Negative calves | Rate | Risk ratio | Odds ratio | Significance |
|---|---|---|---|---|---|---|---|
| North | HPDC (72)APDC (94) | n=21n=33 | n=51n=61 | 0.290.35 | 0.83(0.52-1.3)* | 0.76(0.39-1.47)* | 0.26 |
| South | HPDC (58)APDC (92) | n=17n=40 | n=41n=52 | 0.290.43 | 0.67(0.42-1.07) | 0.53(0.26-1.08) | 0.05 |
| East | HPDC (69)APDC (105) | n=16n=31 | N=53N=74 | 0.230.29 | 0.78(0.46-1.32) | 0.72(0.35-1.44) | 0.22 |
| west | HPDC (68)APDC (103) | n=19n=33 | N=49N=70 | 0.270.32 | 0.87(0.54-1.4) | 0.82(0.42-1.61) | 0.34 |

*95% confidence interval

HPDC: High producing dairy cows

APDC: Average producing dairy cows

Table 3: Epidemiological parameters of rotavirus affected HPDC and APDC diarrheic Holstein calves in dams of different ages

| Years | N0. of calves | Rotavirus positive calves | Rotavirus Negative calves | Rate | Risk ratio | Odds ratio | Significance |
|---|---|---|---|---|---|---|---|
| >2 | HPDC (n=96)APDC (n-142) | n=30n=60 | n=66n=82 | 0.310.42 | 0.73(0.51-1.05)* | 0.62(0.36-1.07)* | 0.05 |
| 3 | HPDC (n=82)APDC (n=125) | n=25n=37 | n=57n=88 | 0.300.29 | 1.03(0.67-1.57) | 1.04(0.56-1.91) | 0.5 |
| ≥4 | HPDC (n=89)APDC (n=127) | n=23n=35 | N=66N=92 | 0.250.27 | 0.93(0.59-1.47) | 0.91(0.49-1.69) | 0.45 |

*95% confidence interval

HPDC: High producing dairy cows

APDC: Average producing dairy cows

Table 4: Epidemiological parameters of rotavirus affected HPDC and APDC diarrheic Holstein calves of different herd size groups

| Herd size | N0. of calves | Rotavirus positive calves | Rotavirus Negative calves | Rate | Risk ratio | Odds ratio | Significance |
|---|---|---|---|---|---|---|---|
| Small | HPDC (81)APDC (141) | n=26n=49 | n=55n=92 | 0.320,37 | 0.85(0.58-1.26)* | 0.79(0.44-1.42)* | 0.26 |
| Medium | HPDC (88)APDC (116) | n=24n=35 | n=64n=81 | 0.270.30 | 0.9(0.58-1.4) | 0.86(0.46-1.6) | 0.38 |
| Large | HPDC (98)APDC (137) | n=30n=46 | N=68N=91 | 0.30.33 | 0.91(0.62-1.33) | 0.87(0.5-1.52) | 0.36 |

*95% confidence interval

HPDC: High producing dairy cows

APDC: Average producing dairy cows

## DISCUSSION

Although the apparent prevalence may underestimate the true prevalence of the disease, it is useful as a consistent index. 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 rotavirus infection in diarrheic fecal samples of HPDC and APDC calves in this study represents the degree of accuracy of ELISA test for rotavirus antigen detection in fecal samples used. Commercial ELISAs are being used increasingly to detect enterophathogens in fecal samples from calves and they have the advantage of not requiring special equipment or expertise and, therefore, they are suitable for small laboratories [13]. The current study showed that the diarrheic APDC and HPDC calves in southern region of Fars province experienced more episodes of rotavirus infection than other regions. There are three distinct climatic regions in the Fars Province of Iran. First, the mountainous area of the north and northwest with moderate cold winters and mild summers. Secondly, the central region, 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. In some parts of this latter area the temperature occasionally rises above 40 degree centigrade in summers. The absorption of Ig may be affected by the environment in which the calf is born [14]. Extreme cold [15] but not moderate cold [16, 18], 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 [15, 18] as well as the ability of the calf to stand and nurse [17, 18]. M0st of fecal samples taken in this area were obtained during summer and it seems that heat stress in southern region of Fars province probably had markedly affected colostral composition and Ig content in HPDC and APDC rotavirus 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 [1]. It was described that that colostrum yield was not reduced when Holstein heifers were exposed to high ambient temperature [19]. However, total fat, lactose, energy, CP, IgG and IgA were lower than those for heifers maintained in a thermoneutral environment [19]. It was also noted that under the same conditions, diarrheic rotavirus infected APDC calves in southern region of Fars province were at much 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. Thee 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 travels through the blood to the adrenal cortex, where cells of the zona fasciculata secrete glucocorticoids [20], with cortisol being the primary glucocorticoid in swine and cattle [21]. Stress hormones released in response to activation of the HPA axis (CRF, ACTH and cortisol) have all been shown to have an effect on aspects of the immune system [22]. It has been shown that incubation of cattle and porcine immune cells with cortisol suppresses lymphocyte proliferation, IL-2 production and neutrophil function [23, 24]. The role of possible different genetic composition of HPDC and APDC rotavirus 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. It was reported that Angus and Braham x Angus cattle responded immunologically differently to shipping stress [25]. Angus calves had greater total IgG and IgM titers against pig red blood cells compared with Simmental calves [26]. Large White pigs had greater post stress ACTH levels after exposure to a novel environment than did Meishan pigs [27]. Studies by [28, 29] 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 [30, 31]. This fact was also demonstrated in the current study and the rate of rotavirus infection in diarrheic Holstein calves was higher in cows between 2 and 3 years old than other older age groups. Furthermore, it was shown that the rate of rotavirus infection in HPDC diarrheic calves in different age groups was lower than APDC calves. This fact may also be related to the better quality and higher clostrum yield in dams of HPDC calves and the better specific and nonspecific immunity provided for them. In conclusion, the current study revealed that there are differences of rotavirus infection in diarrheic HPDC and APDC calves. The rate of rotavirus infection in diarrheic HPDC and APDC Holstein calves can be affected by geographical location and parity of the dam and it was found that the sensitivity of HPDC diarrheic calves to rotavirus infection were lower in these conditions. The lower rate of rotavirus 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 HPDC calves and higher clostrum yield in their dam.

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## REFERENCES

1. Gunn, A., J.A. Naylor and J.K. House, 2009. Diarrhea, In: Large Animal Internal Medicine, Smith, B.P., 4th Edi, C.V. Mosby, pp: 340-354.
2. Bendali, F., M. Sanaa, H. Bichet and F. Schelcher, 1999. Risk factors associated with diarrhoea in newborn calves. Vet. Res., 30: 509-522.
3. 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.
4. Glass, R.I., J. Bresee, B. Jiang, J. Gentsch, T. Ando, R. Fankhauser, J. Noel, U. Parashar, B. Rosen and S.S. Monroe, 2001. Gastroenteritis Viruses: An Overview. Novartis Found. Symp., 238: 5-19.
5. Lorrot, M. and M. Vasseur, 2007. How do the rotavirus NSP4 and bacterial enterotoxins Lead Differently to Diarrhea. Virol. J., 4: 31.
6. Gray, J. and U. Desselberger, 2000. Rotaviruses: Methods and Protocols. Humana Press, pp: 1-2.
7. Duman, R. and A. E.Aycan, 2010. Prevalence of Rotavirus Infections in Calves with Diarrhea in Konya Region. J.A.V.A., 9: 136-138.
8. Theil, K.W., 1990. Group A rotavirus. In: Viral Ddiarrheas of Mman and Aanimals, Editors., Saif L.J. and K.W. Theil, CRC Press, Florida. pp: 35-73.
9. Saif, L.J., B Rosen and A Parwani, 1994. Animal Rrotaviruses. In: Virus Iinfection of Tthe Ggastrointestinal Ttract, Editors., Kapikian A.Z., Marcel Dekker, Inc., New York, pp: 279-367.
10. 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.
11. 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.
12. Rogan, W.J. and J. Gladen, 1978. Estimating prevalence from the results of screening test. Am. J. Epidemiol., 107: 71-76.

13. 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. Preventive. Vet. Med., 7: 145-152.
14. Quigley, J., 2007. Passive Immunity in Newborn Calves. WCDS Adv. Dairy Tech., 19: 247-265.
15. 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.
16. 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.
17. Olson, D.P., L.F. Woodward, R.C. Bull and D.O. Everson, 1981. Immunoglobulin levels in serum and colostral whey of protein metabolizable energy restricted beef Cows. Res. Vet. Sci., 30: 49.
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.
19. 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.
20. Fulford, A.J. and M.S. Harbuz, 2005. An Iintroduction to Tthe HPA. In Handbook of Stress and the Brain, Editors., Steckler, T., N.H. Kalin and J.M.H.M. Reul, Elsevier, Amsterdam., pp: 43-66.
21. 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.
22. Salak-Johnson, J.L. and J.J. McGlone, 2007. Making sense of apparently conflicting data: stress and immunity in swine and cattle. J Anim. Sci., 85: E81-8.
23. Salak-Johnson, 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.
24. Blecha, F. and P.E. Baker, 1986. Effect of cortisol *in vitro* and *in vivo* on production of bovine interleukin 2. Am. J. Vet. Res., 47: 841-845.
25. 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.
26. Engle, T.E., J.W. Spears, T.T. Jr. 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.
27. 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.
28. 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.
29. 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.
30. Kruse, V., 1970. Yield of colostrum and immunoglobulin in cattle at the first milking after parturition. Anim. Prod., 12: 619-626.
31. Roy, J.H.B., 1990. The Calf. Vol. I. Management of Health. Butterworths, pp: 53-117.