Original Article

Bovine immunodeficiency virus and bovine leukemia virus and their mixed infection in Iranian Holstein cattle

Gholamreza Nikbakht Brujeni[1], Taghi Taghi Poorbazargani[2], Susan Nadin-Davis[3], Mohammad Tolooie[4] and Neda Barjesteh[1]

[1]*Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*
[2]*Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*
[3]*Ottawa Laboratory Fallowfield, Canadian Food Inspection Agency, Ottawa, ON, Canada*
[4]*Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran*

Abstract

Introduction: Bovine immunodeficiency virus (BIV) and bovine leukemia virus (BLV) have worldwide distributions, but their prevalences in Iran are unknown. We investigated the presence of infections in Iranian Holstein cattle and determined changes in hematological values for infected animals.

Methodology: Nested PCR was used on blood samples from 143 animals Holstein cattle to detect proviral BIV and BLV gag sequences. Flow cytometric analysis was performed using monoclonal antibodies (mAbs) against CD4, CD8, and CD21 bovine T lymphocyte subsets.

Results: Proviral BIV and BLV gag sequences were detected in 20.3% and 17% of the animals, respectively. BIV-BLV confection was also detected in 4.2% of the study population but this was not statistically significant. Flow cytometric analysis showed that both BIV-infected cows and non-infected ones had CD4/CD8 ratios of 2.45 and 1.43, respectively, and this difference was significant. BLV infected and non-infected animals had no significant differences in their CD4/CD8 ratio. In comparison to non-infected cattle, those with both BIV and BLV had a significant decrease in their CD4/CD8 ratios (1.5 % vs. 2.3; P = 0.01).

Conclusion: This is the first report of BIV and BLV infections in Iran. We found no evidence that infection with one agent predisposed an animal to infection with the other. BIV infection may have a role in decreasing T CD8 counts, but this may depend on the genetics of the cattle and virus strains involved.

Key words: bovine immunodeficiency virus; bovine leukemia virus/ nested PCR; flow cytometry; Iran

J Infect Dev Ctries 2010; 4(9):576-579.

(Received 29 November 2009 – Accepted 03 June 2010)

Introduction

Bovine immunodeficiency or immunodeficiency-like virus (BIV) is a lentivirus of the family Retroviridae. It occurs widely with infections being lifelong and generally subclinical [1,2]. In some instances, however, infections may be of economic importance with seropositive cows having decreased milk production [3-5]. Also, there is evidence that BIV can cause immunosuppression with increased incidences of secondary bacterial infections and encephalitis found in herds with high seroprevalences [4-9]. Following experimental infections, cattle may have transient increases in lymphocytes, lymphoid hyperplasia, atypical lymphosarcoma, and secondary bacterial infections [6,10,11]. It has also been suspected that the stress of parturition in BIV infected cows is associated with the progression of bovine leukemia virus (BLV) infections [8,12,13]. The clinical significance of BIV infection may depend upon the strain of BIV, the breed of cattle, and environmental stressors [2,6].

Bovine leukaemia virus (BLV) is a lymphotropic retrovirus structurally related to human T-cell leukaemia virus type 1 (HTLV-1) [14]. Following infection, the BLV can remain clinically dormant, cause a persistent lymphocytosis (PL) with increased B lymphocytes, or cause B-cell lymphomas in lymph nodes and other tissues. Only a few (< 5%) BLV-infected animals develop tumors [14].

As there is no data on BIV and BLV in Iran, we conducted a survey of dairy cattle to determine the prevalence of single and concurrent infections. We also determined the hematological effects of infections.

Methodology

Whole blood and blood in EDTA were collected from 143 apparently healthy Holstein cows in Maysam slaughterhouse (Tehran, Iran). Complete blood counts were established by routine procedures in the Tehran University laboratory.

Single-colour staining for flow cytometric analysis was performed using the following commercially available mAbs against bovine lymphocyte subsets (Serotec, Kidlington, England): CD4- fluorescein isothiocyanate (FITC), CD8-FITC and CD21- FITC. Mouse IgG1-FITC and IgG2a- were used as negative controls.

Peripheral blood cells were lysed (DakoCytomation Uti-Lyse Erythrocyte Lysing Reagent, Dako, Glostrup, Denmark) and lymphocytes were labeled using the mAbs according to the manufacturer's instructions. Flow cytometric analysis was performed by counting 10,000 cells from each sample on a FACS flow cytometer (Becton–Dickinson and Co. Immunocytometry Systems, Franklin Lakes NJ, USA) and analyzed with CELLQuest software.

For the preparation of DNA, aliquots of whole blood (500 µl) were added to 1 ml red blood cell lysis buffer (10 mM Tris-HCl pH 7.6; 5 mM MgCl2, 100 mM NaCl, 0.75 %Triton X-100) mixed and incubated at room temperature. After 2 minutes the tube was centrifuged at 12000 g for 20 seconds and supernatant was discarded. The pellet was resuspended in 600 µl of cell lysis buffer (10 mM Tris-HCl, pH 8; 0.1 M NaCl, 25 mM EDTA, pH 8; 0.5% sodium dodecyl sulphate) and homogenized. Then 300 µl denaturing solution containing 4M guanidinium isothiocyanate, 25 mM sodium citrate pH 7; 0.1 m 2ME and 0.5 % Sarkosyl was added and incubated at room temperature. After 10 minutes, 300 µl potassium acetate (3M potassium and 5M acetate) were added and vortexed for 30 seconds. After centrifugation for 3 minutes at 12000g, 900 µl supernatant were recovered and DNA precipitated with 600 µl isopropanol. The pellet was washed with 70% ethanol and resuspended in 50 µl of TE (pH, 7.6). All samples were stored at -20°C until used.

The DNA extracted from each blood sample was used as a template to detect BIV proviral DNA by nested PCR as described previously by Nadin-Davis *et al.* [17]. Plasmid DNA containing the complete BIV gag coding region (pGEM7-gag kindly provided by S. Nadin-Davis) served as a positive control. DNA from the animals that was consistently assayed as BIV negative was used as negative control. Nested PCR was also used to detect BLV proviral DNA as described previously by Wang *et al*. [18]. DNA extracted from BLV-infected FLK cells (kindly provided by Razi Institute, Iran) was used as a positive control and the DNA sample prepared from a BLV-seronegative cow was used as negative control for PCR amplification.

PCR products generated from three BIV positive samples were cloned using a QIAGEN PCR cloning kit (QIAGEN, Hilden, Germany) and sequenced commercially (Macrogen Inc., Seoul, Korea). Sequences were aligned and phylogenetic analyses performed with the Clustal programme available in the software package of Bioedit version 7.0.5.3 (http://jwbrown.mbio.ncsu.edu/Bioedit/bioedit.html). Sequences obtained were deposited in GenBank (EF661978, EF661979 and EF661980).

Data obtained from hematological and flow cytometric experiments were analyzed by the SPSS 15.0 program using chi-square and Student's t test.

Results

Nested PCR identified 29 BIV-positive samples giving an overall positivity rate of 20.3% (29/143). Similarly, 16.8% (24/143) were positive with the BLV-gag nested PCR. Six animals were positive for both viruses (6/143; 4.2 %).

There was 98-100% identity when sequences of the Iranian BIV amplicons (EF661978, EF661979 and EF661980) were compared with those of isolate R-29, confirming that BIV was detected in the Iranian cattle we studied using our BIV specific primers. The sequences had no similarity with the bovine genome and other known related organisms (JDVCG).

The only significant differences found, when comparing hematological and flow cytometery results, were the proportions of CD8 cells in BIV infected and non infected animals (27% and 21%; $P \leq 0.05$); the number of BIV and BLV infected animals (26% and 17%; $P \leq 0.5$); and the proportions of CD4 cells in BLV infected and non infected animals (36% and 30%; $P \leq 0.05$). When CD4/CD8 ratios were calculated, significant differences were also observed between BIV infected and non infected animals (1.4 and 2.4; $P \leq 0.05$) and BIV-BLV coinfected animals (1.5 and 2.3; $P \leq 0.5$).

Discussion

Although BIV infected cattle have been identified in some countries neighboring Iran [4,19], to the best of our knowledge this is the first report of

BIV infection in Iranian Holstein cattle. Although we studied only a small number of animals, the prevalence we found of 20.3% is similar to that reported from other Asian countries (12% in Turkey [19] to 35% in Korea [20]), and neighboring countries such as Pakistan (16 %) [4,13]. The prevalence of BLV infection (17%) in the current study was also similar to that of other Asian and non Asian countries (9 to 33 %) [21-24].

There was a significant increase in the CD4/CD8 ratio in BIV-infected cows due to a significant decrease in CD8 counts. Previous studies have shown there are no significant changes in lymphocyte counts and CD4/CD8 ratios [3,7,9,28,29] and increased [9] CD8 counts. It is unclear why such differences occur, but immune dysfunction and/or some changes in leukocyte proportions might be present only temporarily after BIV infection [9] and concurrent BIV and BLV infection may play an important role.

Our study showed BIV infection is more common than BLV infection in the Iranian cattle we studied and we found no evidence that infection with one virus predisposed to infection with the other. This is consistent with the results reported by Meas (2003) [19] although González *et al*. and Meas *et al*. (2002) showed there was a statistical correlation between BIV and BLV infections [15,30]. The reasons for these different results have not been examined but may reflect genetic differences between the cattle and the virus types involved in the studies.

Our study adds to the available data on BIV and BLV and is the first report of the diseases in Iran. Further studies are indicated to determine the epidemiology of infections in Iran, and local dairy farmers need to be informed of the health risks these infections pose to their animals.

References

1. Andrews AH, BlowyW, Boyd H, Eddy RG (2004) Bovine Medicine, second edition. Oxford, UK: Blackwell Science 693-700 pp.
2. Radostitis OM, Done SH, Blood DC (2007) Veterinary Medicine a textbook of the diseases of cattle, horses, sheep, pigs and goats, 10th edition. London: Saunders Elsevier 1046-1058, 1213, 1221-1223 pp.
3. Cavirani S, Donofrio G, Chiocco D, Foni E, Martelli P, Allegri G, Cabassi CS, De IB, Flammini CF (1998) Seroprevalence to bovine immunodeficiency virus and lack of association with leukocyte counts in Italian dairy cattle. Prev Vet Med 37: 147-157.
4. Meas S, Seto J, Sugimoto C, Bakhsh M, Riaz M, Sato T, Naeem K, Ohashi K, Onuma M (2000) Infection of bovine immunodeficiency virus and bovine leukemia virus in water buffalo and cattle populations in Pakistan. J Vet Med Sci 62: 329-331.
5. Snider TG, III, Hoyt PG, Coats KS, Graves KF, Cooper CR, Storts RW, Luther DG, Jenny BF (2003) Natural bovine lentiviral type 1 infection in Holstein dairy cattle. I. Clinical, serological, and pathological observations. Comp Immunol Microbiol Infect Dis 26: 89-101.
6. Belloc C, Polack B, Schwartz-Cornil I, Brownlie J, Levy D (1996) Bovine immunodeficiency virus: facts and questions. Vet Res 27: 395-402.
7. Isaacson JA, Flaming KP, Roth JA (1998) Effects of long-term infection with bovine immunodeficiency virus and/or bovine leukemia virus on antibody and lymphocyte proliferative responses in cattle. Vet Immunol Immunopathol 64: 249-266.
8. Snider TG, III, Coats KS, Storts RW, Graves KF, Cooper CR, Hoyt PG, Luther DG, Jenny BF (2003) Natural bovine lentivirus type 1 infection in Holstein dairy cattle. II. Lymphoid tissue lesions. Comp Immunol Microbiol Infect Dis 26: 1-15.
9. Whetstone CA, Suarez DL, Miller JM, Pesch BA, Harp JA (1997) Bovine lentivirus induces early transient B-cell proliferation in experimentally inoculated cattle and appears to be pantropic. J Virol 71: 640-644.
10. Hirai N, Furuyama H, Awaya A, Onuma M (1995) Effect of administration of serum thymic factor (FTS) in calves and rabbits infected with bovine immunodeficiency-like virus. J Vet Med Sci 57: 307-310.
11. Walder R, Kalvatchev L, Perez F, Garzaro D, Barrios M (2001) Bovine immunodeficiency virus in experimentally infected rabbit: tropism for lymphoid and nonlymphoid tissues. Comp Immunol Microbiol Infect Dis 24: 1-20.
12. Meas S, Ohashi K, Tum S, Chhin M, Te K, Miura K, Sugimoto C, Onuma M (2000) Seroprevalence of bovine immunodeficiency virus and bovine leukemia virus in draught animals in Cambodia. J Vet Med Sci 62: 779-781.
13. Usui T, Meas S, Konnai S, Ohashi K, Onuma M (2003) Seroprevalence of bovine immunodeficiency virus and bovine leukemia virus in dairy and beef cattle in hokkaido. J Vet Med Sci 65: 287-289.
14. Murphy FA, Gibbs EPJ, Horzinek MC, Studdert MJ (1999) Veterinary virology, 3th edition. San Diego: Academic Press 363-389 pp.
15. Meas S, Ruas J, Farias NA, Usui T, Teraoka Y, Mulenga A, Chang KS, Masuda A, Madruga CR, Ohashi K, Onuma M (2002) Seroprevalence and molecular evidence for the presence of bovine immunodeficiency virus in Brazilian cattle. Jpn J Vet Res 50: 9-16.
16. Jacobs RM, Pollari FL, McNab WB, Jefferson B (1995) A serological survey of bovine syncytial virus in Ontario: associations with bovine leukemia and immunodeficiency-like viruses, production records, and management practices. Can J Vet Res 59: 271-278.
17. Nadin-Davis SA, Chang SC, Smith H, Jacobs RM (1993) Detection of bovine immunodeficiency-like virus by the polymerase chain reaction. J Virol Methods 42: 323-336.
18. Wang H, Norris KM, Mansky LM (2002) Analysis of bovine leukemia virus gag membrane targeting and late domain function. Journal of Virology 76: 8485-8493.
19. Meas S, Yilmaz Z, Usui T, Torun S, Yesilbag K, Ohashi K, Onuma M (2003) Evidence of bovine immunodeficiency virus in cattle in Turkey. Jpn J Vet Res 51: 3-8.
20. Cho KO, Meas S, Park NY, Kim YH, Lim YK, Endoh D, Lee SI, Ohashi K, Sugimoto C, Onuma M (1999)

Seroprevalence of bovine immunodeficiency virus in dairy and beef cattle herds in Korea. J Vet Med Sci 61: 549-551.
21. Batmaz, H., Carli, K.T., Kahraman, M., Kennerman, E (1995) Serological and hematological diagnosis of enzootic bovine leucosis in cattle in Turkey. Vet Rec 36: 42-44.
22. Johnson R, Kaneene JB (1992) Bovine leukemia virus and Enzootic bovine leukosis.Vet. Bull. 62: 287.
23. Trono KG, Perz-Filgueira DM, Duffy S, Borca MV, Carrillo C (2001) Serprevalence of bovine leukemia virus in dairy cattle in Argentina: comparison of sensitivity and specificity of different detection methods. Vet Microbiol 83: 235-248.
24. Vanleeuwen JA, Tiwari A, Plaizier JC, Whiting TL (2006) Seroprevalence of antibodies against Bovine leukemia virus, Bovine viral diarrhea, Mycobacterium avium Para tuberculosis and Neospora caninum in beef and dairy cattle in Manitoba. Can Vet J 47: 783-786.
25. Van der Maaten MJ, Boothe AD, Seger CL (1972) Isolation of a virus from cattle with persistent lymphocytosis. J Natl Cancer Inst 49: 1649-1657.
26. Jacobs RM, Jefferson BJ, Suarez DL (1998) Prevalence of bovine immunodeficiency-like virus in bulls as determined by serology and proviral detection. Can J Vet Res 62: 231-233.
27. Zhang S, Wood C, Xue W, Krukenberg SM, Chen Q, Minocha HC (1997) Immune suppression in calves with bovine immunodeficiency virus. Clin Diagn Lab Immunol 4: 232-235.
28. Onuma M, Koomoto E, Furuyama H, Yasutomi Y, Taniyama H, Iwai H, Kawakami Y (1992) Infection and dysfunction of monocytes induced by experimental inoculation of calves with bovine immunodeficiency-like virus. J Acquir Immune Defic Syndr 5: 1009-1015.
29. Suarez DL, Whetstone CA (1997) Comparison of different PCR tests to detect bovine lentivirus in cell culture and experimentally and naturally infected cattle. J Vet Diagn Invest 9: 421-424.
30. González ET, Licursi M, Vila Roza V, Bonzo E, Mortola JP, Frossard JP, Venables C (2008) Evidence of bovine immunodeficiency virus (BIV) infection: Serological survey in Argentina. Res Vet Sci 85: 353-358.

Corresponding author

Gholamreza Nikbakht Brujeni
Faculty of Veterinary Medicine
University of Tehran
Qareeb Street
Azadi Av.Tehran- Iran
Tel: (98) (21) 61117057; Fax: (98) (21) 66427517
Email: nikbakht@ut.ac.ir

Conflict of interests: No conflict of interests is declared.