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Molecular prevalence for Bovine immunodeficiency virus infection in Iranian cattle population

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Bovine immunodeficiency virus (BIV), a member of the family Retroviridae, is an infectious pathogenic lentivirus in cattle. Although, BIV induced cattle infections are reported in several countries of the world, its prevalence in Iran is not clearly known. In this investigation, we report the detection of proviral DNA sequence of BIV in 300 blood samples of cattle by polymerase chain reaction (PCR) using oligonucleotiode primers specific for the *gag gene* region of the virus. Blood samples were taken from Chaharmahale Bakhtiary province. According to the PCR results, infection rate in the cattle population were 60%. This is the first report for the presence of BIV in cattle and sheep population of Chaharmahale Bakhtiary province, and the first evidence for sheep infection.

Key words: Bovine immunodeficiency virus, cattle, Chaharmahale Bakhtiary province, Iran, PCR.

INTRODUCTION

Lentiviruses are a widely dissiminated group of exogenous non oncogenic retroviruses which include visna-maedi virus of sheep, equine infectious anaemia virus (EIAV), caprine arthritis encephalitis virus (CAEV), bovine immunodeficiency virus (BIV), feline immuno-deficiency virus (FIV) and jembrana disease virus (JDV) (Patil et al., 2003). These viruses are genetically related and share certain biologic and pathogenic characteristics. There is also cross reactivity between antigens of different lentiviruses (St-Louis et al., 2004; Sinder et al., 2003; Horzinc et al., 1991).

Since BIV recognition as a lentivirus in the late 1980s, BIV infections have been shown to be prevalent glo-bally and variably associated with alterations in animal production, weight loss, secondary disease, diminished milk production and increased incidence of encephalitis (Gradil et al., 1999; Tajima et al., 1997; Flaming et al., 1993). Although, BIV induces dysfunction of monocytes and neutrophils, BIV inoculated calves did not exhibit severe clinical symptoms, and pathogenesis of BIV in cattle remains unclear (Carpenter et al., 2000; Gonzale et al., 2000; Yilmaz et al., 2008). Even though the virus has not been linked to any specific disease condition in cattle, it certainly can aggravate certain illnesses in the animals, including impairment of the immune system (Carpenter et al., 2000).

The mature form of BIV is bare/core shaped and 120 - 130 nm in diameter (St-Louis MC et al., 2004; Narayan et al., 1989). In general, lentiviruses genome offers a com-plex structure including several regulatory/accessory *genes* that encode proteins, some of which are involved in the regulation of virus *gene* expression (St- Louis et al., 2004). There are difficulties in the isolation of the BIV

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Abbreviations: EIAV, Equine infectious anaemia virus; CAEV, caprine arthritis encephalitis virus; BIV, bovine immunodeficiency virus; FIV, feline immunodeficiency virus; JDV, jembrana disease virus; PCR, polymerase chain reaction; EDTA, ethylene diamine tetra acetic acid.

from field cases. Attempts to culture BIV from cattle have been unsuccessful (Patil et al., 2003). Two approaches have often been used to detect BIV: the direct fluorescent antibody assay (IFA) and the nested PCR. IFA detects an antibody; whereas, PCR detects the provirus genome, (Orr et al., 2003; Zhang et al., 1997). Even though BIV infection is an emerging disease of cattle world wide, its status in Iran is not known. The present study was undertaken to look for the possible presence of proviral genomic sequence of the virus (BIV) in randomly collected blood samples of cattle in Chaharmahale Bakhtiary province.

MATERIALS AND METHODS

Blood sampling and DNA extraction

A total of 300 whole peripheral blood samples containing ethylene diamine tetra acetic acid (EDTA) anticoagulant agent were randomly collected from cattle in Chaharmahale Bakhtiary province of Iran in early 2009. All animals were clinically normal and older than one year of age at the start of this study. The blood samples (10 ml) were centrifuged at 3,000 rpm for 35 min at 18°C. Buffy coat cells were resuspended in 4 volumes of sterile 0.2% NaCl to lyse erythrocytes. After l min, 7.2% NaCl was added to reconstitute isotonicity and stored at -20°C until further use (Muller-Doblies et al., 1998).

Extraction of DNA

DNA was extracted from buffy coat as previously described by Muller - Doblies et al., 1998. Briefly, lyses buffer (Tris-HC1 100 mM, EDTA 0.5 M, sodium dodecyl sulfate 10X, NaCl 5 M, and proteinase k 20 mg/ml, 500 µl) was added to buffy coat and incubated at 37°C for 2 – 3 h .Then, 100 µl of phenol, 96 µl chloroform, and 4 µl isoamyl alcohol were added to 200 µl of prepared incubated buffy coat cells. Mixture was centrifuged at 11,000 rpm for 4 min. From the three phases after centrifugation, the supernatant was isolated and equal volume of ethanol (100%) and congregation at 4,000 rpm for 5 min. DNA pellet was washed with 1 ml of ethanol (75%) and the mixture was centrifuged at 7,000 rpm for 5 min and DNA was dried on air. DNA concentration was determined by measuring the optical density (OD) at 260 nm by spectrophotometer (Eppendorf Biophotometer Instrument, Germany).

Polymerase chain reaction

To detect the BIV provirus DNA, PCR was performed using the DNAs extracted from puffy coat samples. All samples were examined by PCR using the method of Gonzalez et al., 2000. The primers chosen had the following sequences:

BIV-*gag*-F: 5'-GGATCCGAGGCCAGAGCTGATAAGGAA-3' (652-672)
BIV-*gag*-R: 5'- CTCGAGATCCCACTACCCTACATGCT-3' (1374-1393)

All amplification reactions were performed in a 25 µl deoxy ribonucleotide triphosphates 1.25 mM, *3* µl forward primer, 3 µl reverse primer, 0.3 µl smart Taq DNA polymerase 5 u (Roche Applied science, Germany), 0.2 µl template DNA, and 10.7 µl D.D.

water. Genomic DNA derived from buffy coat sample was used as template. BIV genome was amplified by 1 cycle (95°C for 1 min), 30 cycles (95°C for 1 min , 55°C for 1 min and 72°C for 2 min) and 1 cycle (72°C for 10 min) using oligodeoxy nucleotides for the *gag gene*. Fifteen microliters of each reaction mixture was mixed with 3 µl of loading buffer 6× and run on a 1% agarose gel for evaluation by ethidiume bromide visualization in gel electrophoresis by gel documentation (Uvitec UK compony), and 1000 bp DNA marker (fermentase) was used to distinguish DNA fragment bands in lanes. Plasmid DNA containing the complete BIV *gag* coding region (pGEM7-gag) served as a positive control for PCR amplification (Nadin-Davis et al., 1993). Water was used as a negative control.

RESULTS

The PCR assay was able to detect BIV proviral DNA from 300 samples of cattle's by using primers mentioned in materials and methods. The existence of 754 bp figment in samples showed positive PCR assay. Of the 300 DNA samples from cattle's, 180 specimens (60%) contained 754 bp DNA fragment bands. The results are shown in Table 1 and Figure 1.

DISCUSSION

Results of many studies concerning human immunodeficiency virus (HIV) have provided the widespread field for studying genetic variability, laboratory diagnosis, epidemiological studies and finally appropriate strategies for preventing retrovirus infectious. Among cattle retroviruses, the Bovine immunodeficiency virus is significant; therefore, many widespread researches have been made on diagnosis, control and prevention methods for this disease is by using serological and molecular biologic methods.

The implementation of standardized PCR testing for BIV warrants further rigorous examination of the sensitivity and specificity of the assay. Earlier studies indicated discordance between serological and genomic detection of BIV, with genomic detection by polymerase chain reaction showing greater sensitivity and specificity (Gonzale et al., 2000) have provided evidence that their nested PCR has a greater sensitivity than other published methods.

This is the first report of molecular evidence for BIV infection in cattle in Chaharmahale Bakhtiary province. There were not any previous reported concerning BIV infections in cattle in Chaharmahale Bakhtiary Provience. Seroepidemiological investigations have revealed that, BIV infection may be common in both beef and dairy cattle (St.- Cyr Coat et al., 1994). BIV can induce dysfunction of monocytes and neutrophils but did not exhibit severe clinical symptoms, so, pathogenesis of BIV in cattle remains unclear .There are some report regarding the prevalence of BIV in the world. An early report of the incidence of BIV in Louisiana cattle indicated a collective seroprevalence of 11% in four dairy herds

Table 1. Statistic data of the tested animals.

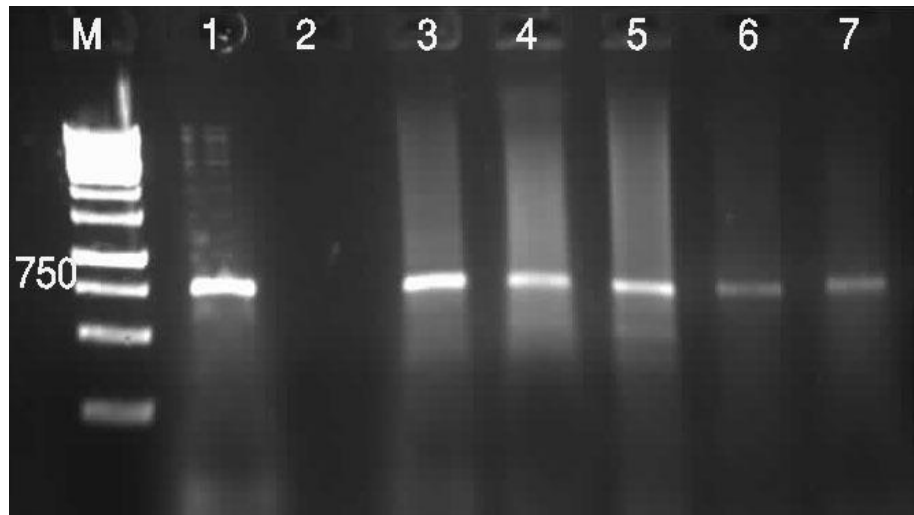| Positive ratio	Positive specimen	Samples	Species
60%	180	300	Cattle

Figure 1. Ethidium bromide stained agarose gel of PCR products amplified with BIV gag primers. DNA I000 bp markers (lane M), Positive control (lane1), Negative control (lane 2), DNA samples from cattle (lane 3-7).

(Amborski et al., 1989) but in 1992 BIV seroprevalence of 40% in beef herds and 64% in dairy herds was detected in Louisiana cattle (Gonda et al., 1992).

Cockerell et al. (1992) reported a 21% seroprevalence of BIV in a Colorado dairy herd. In (1998), Cavirani et al. reported 5.8% seropositivity in Italian dairy cattle's. St.-Cyr Coat et al. (1994) examined the seroepidemiology of BIV infection in two Mississippi dairy herds; (coastal plains and MSU). Serology revealed a 38% incidence of BIV infection in coastal plains animals and 58% incidence in MSU animals. This investigation indicates that BIV infection is prevalent in Mississippi animals. Carpenter et al. (1999) reported higher circulating lymphocytes and follicular hyperplasia of lymph nodes, hemal nodes and spleen in calves experimentally infected with BIV within 6 weeks postinoculation.

Gonzale et al. (2000) utilized a simple *gene* amplification technique for detection of sequences from the 3 major BIV *genes*, *gag*, *pol* and *env*. They indicated that, the frequency of BIV infection is 5.5 - 12% among dairy cattle in Ontario but elsewhere in North America, the frequency is highest. Many studies by Meas et al. (2000) have been performed in different parts of the world with the used of serological methods, using recombinant p_{26} protein BIV. A study conducted in 1998 through western blot method revealed that 11.7% of the cattle's in Hokkaido had the antibodies against BIV. In a study performed in 5 states in Cambodia in 2000, 544

cattle and 42 buffalos were examined in this study 26.3% of the buffalos, respectively, positive for anti BIV- P_{26} antibodies by western blotting. In other survey, Meas et al. (1999) reported that 10.3% seropositivity in buffalo and 15.8% in cattle in Pakistan by using recombinant nested PCR assay to detect proviral DNA in seropositive buffaloes and cattles. Serological and molecular methods have shown that 12.3% of cattles were infected with BIV in Turkey in 2003.

In 2004 were found antibody against recombinant P26 protein BIV in 11.4% of the cattles in Mubura, Zambia. Animal lentiviruses are similar to their human counterparts in many important aspects of their biology. Because of these biologic similarities and their genetic relationship to human immunodeficiency virus type-1 (HIV- 1), the non human lentiviruses have been recognized as potentially useful models for understanding the pathogenesis of HIV-1 and evaluating methods for effective treatment and control of viral infections (Horzienk et al., 1991; Gonda et al., 1987).

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