

Prevalence of Bovine Immunodeficiency Virus Infection in Buffaloes in East Azerbaijan, Northwestern Iran

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Abstract: Bovine immunodeficiency virus (BIV) has a worldwide distribution, but its prevalence in different regions of Iran is unknown. In this study, for the first time, the presence of BIV infections is detected by using the PCR method in Iranian water buffalo in East Azerbaijan. For this matter, blood samples were taken from 83 randomly selected buffaloes slaughtered in Tabriz industrial slaughterhouse from June to October 2012. All of the animals were clinically examined before sampling. Viral Gene-spin™ Viral DNA/RNA Extraction Kit was used to extract the DNA, and PCR was performed on the extracted DNA using oligonucleotide primers specific for the gag gene region of the BIV virus. The prevalence of BIV in buffaloes was 2.4% (2 of 83), which is less than the prevalence of BIV in Pakistan (10.3%) and India (19%). The low prevalence observed in this study may be due to our small sample size.

Keywords: Bovine immunodeficiency virus, PCR, Buffalo, Iran.

INTRODUCTION

Bovine immunodeficiency virus (BIV) is a lentivirus. Lentiviruses are a widely disseminated 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 immunodeficiency virus (FIV) and jembrana disease virus (JDV). These viruses are genetically related and share certain biologic and pathogenic characteristics [1]. There is also cross-reactivity between antigens of different lentiviruses [2, 3]. BIV is also closely related to the human immunodeficiency virus type 1 (HIV-1) [4, 5].

BIV was originally isolated from an 8-year-old dairy cow with persistent lymphocytosis, progressive weakness and wasting and was appointed in 1972 as R29 [6]. After BIV's recognition as a lentivirus in the late 1980s, it has been shown that the BIV infections occur widely, causing lifelong and generally subclinical diseases [7].

BIV infections have been shown to be variably associated with alterations in animal production, weight loss, secondary diseases, decreased milk production

and increased incidence of encephalitis [8-10]. Whether BIV transmission via uterus, placenta, colostrum, or milk is still under investigation but Proviral DNA of BIV was also detected in bull semen [11, 12]. and it has been shown that the seroprevalence of BIV infection increases according to the aging of animals in the same dairy herd, suggesting that BIV would be possibly transmitted through natural or artificial inseminations, and/or through blood instrument or blood sucking insects [13-15]. Although BIV induces dysfunction in monocytes and neutrophils, BIV inoculated calves did not exhibit severe clinical symptoms, so pathogenesis of BIV remains unclear [16-18]. The clinical significance of BIV infection can depend on the strain of BIV, breed of the cattle, and environmental stressors [19].

Buffalo is a native animal of Iran and East Azerbaijan province, with a total population of about 92620, is one of the most important regions of buffalo farming. As there is no data on BIV in Iranian buffaloes, we conducted the prevalence of this infection in Azerbaijan.

MATERIALS AND METHODS

Blood Sampling

A total of 83 whole peripheral blood samples were collected from randomly selected Asian water buffaloes

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(*Bubalus bubalis*) slaughtered in Tabriz industrial slaughtered house in East Azerbaijan province of Iran from June to October 2012. (31 animals were female and the others were male and the all of them had more than 3-year-old age.) Ethylenediaminetetraacetic acid (EDTA) was used in sampling tubes as an anticoagulant agent.

For the preparation of DNA, aliquots of whole blood (500 μ l) were added to 1 ml red blood cell lysis buffer (10 mM Tris-HCl pH 7.6; 5mM $MgCl_2$, 100mM NaCl, 0.75 % Triton X-100) and were mixed and incubated at room temperature for 2 minutes. The tubes were then centrifuged at 12000g for 20 seconds and the supernatant was discarded. The pellets were frozen at $-70^\circ C$ until they were examined.

DNA Extraction

For DNA extraction, Viral Gene-spin™ Viral DNA/RNA Extraction Kit (Intron Biotechnology.Inc) was used.

At first, the pellet was transferred to the 1.5ml micro-centrifuge tube, resuspended in 250 μ l Viral Gene-spin™ buffer (Lysis buffer) and incubated at $80^\circ C$ for 10 min. After that, it was mixed by vortexing for 15 seconds and incubated at room temperature (15-25 $^\circ C$) for 10 min, then 350 μ l of binding buffer added and completely mixed by gently vortexing, subsequently the solution was placed in a spin column in a provided 2ml collection tube and centrifuged at 13,000 rpm for 1 min. The solution in collection tube was discarded and the column was placed back in the same 2ml collection tube, afterwards, 500 μ l of Washing Buffer A was added to the column and centrifuged for 1 min at 13000rpm, the solution in collection tube was discarded and placed the spin column back in the same 2ml collection tube. Then 500 μ l of Washing Buffer B was added to the column and centrifuged for

1 min at 13000rpm. The solution in collection tube was discarded and the spin column was placed back in the same 2ml collection tube, subsequently centrifuged for 1min at 13000rpm. Then the column was placed in an RNase-free 1.5ml micro-centrifuge tube, 60 μ l of Elution buffer was added directly onto the membrane and was incubated at room temperature for 1 min, then was centrifuged for 1 min at 13000rpm. 2-5 μ l of an eluted solution was used as a template for PCR.

Polymerase Chain Reaction

The DNA extracted from each blood sample was used as a template to detect BIV proviral DNA by PCR as described previously by Nikbakht *et al.* (2010) [20]. The primers chosen are listed in Table 1.

A 25 ml PCR reaction mix contained 0.5 μ l of each primer, 0.2 μ l of dNTP, 0.5 μ l Taq DNA polymerase, 1 μ l $MgCl_2$ (concentration fixed by titration tests), 2.5 reaction buffer, and 16 μ l D.D. water. Two ml of DNA template were obtained from all samples. The PCR was performed for 37 cycles in three stages: 1 cycle ($94^\circ C$ for 1 min, $51^\circ C$ for 45 second, $72^\circ C$ for 1 min), 35 cycles ($94^\circ C$ for 45 second, $51^\circ C$ for 30 second, $72^\circ C$ for 45 second) and 1cycle ($72^\circ C$ for 5 min). 7 μ l of each reaction mixture was mixed with 2 μ l of loading buffer and run on a 1.2% agarose then stained by ethidium bromide and visualized by a U.V. transilluminator. 750bp DNA marker 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 and water was used as a negative control.

RESULTS

Of the 83 DNA samples from buffalo's blood, 2 specimens (2.4%) contained 393bp DNA fragment bands (Table 2).

Table 1: Nucleotide Sequences and Positions of Primers Used for Amplification of gag Fragment of Bovine Immunodeficiency Virus

bp	Sequence	Genomic position	gen	Primer
393	5'- AGATCTGGTCAGACGCCA- 3'	821-838	<i>gag</i>	NIK BIV F
	5'- CTGTGTACGGCTCCTTGG- 3'	1196-1213	<i>gag</i>	NIK BIV R

Table 2: Prevalence of BIV Buffaloes in East Azerbaijan

Species	Samples	Positive samples	Positive ratio
Buffalo	83	2	2.4%

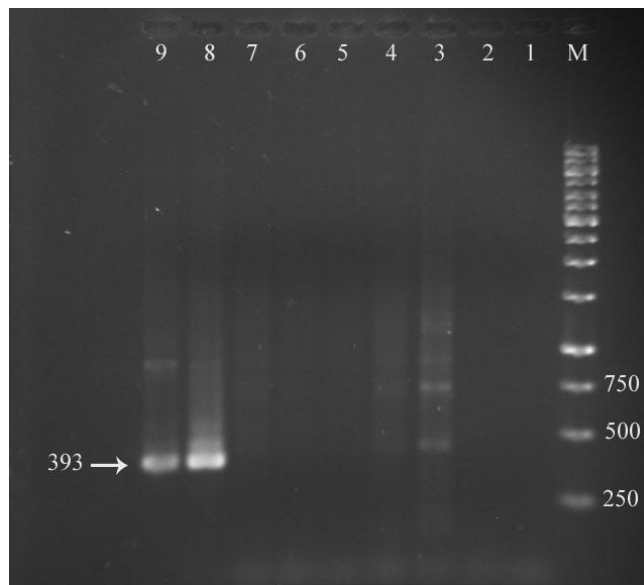


Figure 1: Ethidium bromide-stained agarose gel of PCR products amplified with BIV gag primers. DNA I, 000 bp markers (lane M), negative control (lane 1), positive control (lane 9), DNA samples from Buffaloes (lane 2–8). Sample 3 and 8 are positive.

DISCUSSION

BIV is prevalent globally. The earliest report of the incidence of BIV in Louisiana cattle indicated a collective seroprevalence of 11% in four dairy herds [21] and in 1992 seroprevalence of BIV in beef herds and dairy herds of Louisiana was reported 40% and 60%, respectively [22].

In 1992 BIV seroprevalence was reported 21% in a dairy herd of Colorado [23] and one study in Italy demonstrated that 5.8% of the dairy herds and 2.5% of the tested cows were seropositive for BIV [24]. A seroepidemiological investigation of BIV infection in two Mississippi dairy herds revealed 38 - 58% incidence of BIV infection in [15]. In Canada Gonzalez *et al.* utilized a simple gene amplification technique for detection of sequences from the 3 major BIV genes, gag, pol and env. and indicated that, the frequency of BIV infection is 5.5 - 12% among dairy cattle in Ontario [17]. A study in Argentina showed that 12% of the animals tested were positive for BIV [25]. In 1998 a study using western blot method revealed that 11.7% of the cattle in Hokkaido had the antibodies against BIV [14]. In another study by Meas *et al.* (2000) [26] performed in 5 states in Cambodia, 544 cattle and 42 buffaloes were examined. This research indicated that 26.3% of the cattle and 16.7% of the buffaloes were positive for anti-BIV-P26 antibodies. In another survey that was performed on buffaloes in Pakistan by using recombinant nested PCR assay to detect proviral DNA,

10.3% of buffaloes and 15.8% of cattle were seropositive [11]. In a study in Zambia, 11.4% out of a total of 262 sera were found positive for anti-BIV p26 antibodies [27]. In Korea, 35% and 33% of dairy and beef cattle were BIV seropositive, respectively [13]. A serological and molecular study showed that 12.3% of cattle were infected with BIV in Turkey [18]. A study in India showed that 22% of cattle and 19% of buffaloes were seropositive [28]. In Poland, 1541 serum samples from Holstein cattle from 23 herds were analyzed using ELISA method. The average BIV prevalence was 4.9% in individual cattle while the percentage of herds harboring at least one seropositive animal, was 82.6% [29].

Investigation on BIV in Iran revealed 20.3% of positive cattle in Tehran province [20], 60% and 30% of positive cattle and sheep in Chaharmahal Bakhtiary province of Iran [30].

To the best of our knowledge, this is the first report of BIV infection in Iranian water buffalo.

The prevalence of BIV in buffaloes in this study was lower than the prevalence of BIV observed in Pakistan (10.3%) and India (19%). However, this result may be due to the small sample size. Our study adds to the available data on BIV and is the first report of this disease in buffaloes in Iran. Further studies are needed to determine the epidemiology of the infections in Iran.

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