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NOTE

Evidence of bovine immunodeficiency virus in cattle in Turkey

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Abstract

A seroepidemiological study of bovine immunodeficiency virus (BIV) and bovine leukemia virus (BLV) infections was conducted in four different cattle herds in Turkey. A total of 300 blood samples were analyzed and 12.3% were found to be positive for anti-BIV p26 antibodies by Western blot analysis and 1.6% positive for anti-BLV gp51 antibodies by an immunodiffusion test. BIV infection was confirmed with the detection of BIV-provirus DNA using the nested polymerase chain reaction. This is the first evidence for the presence of BIV in cattle in Turkey.

Key words : bovine immunodeficiency virus ; bovine leukemia virus ; molecular evidence ; seroprevalence ; Turkey.

Bovine immunodeficiency virus (BIV) is a member of the family *Retroviridae*, genus *Lentivirus*. BIV R29 was originally isolated from an 8-year-old dairy cow in Louisiana which was suspected of having lymphosarcoma with persistent lymphocytosis[29]. BIV was characterized in greater detail by Gonda and colleagues[8] who found that structurally, immunologically, and genetically, it more closely resembled the human and non-human primate immunodeficiency viruses. However, conclusive evidence that BIV causes immunodeficiency in cattle has not been established.

Since 1972[29], BIV has been detected in dairy and beef cattle in the USA[26], New Zealand[11], the Netherlands[12], Australia[6], the United Kingdom[5], Canada[13], Germany[20], France[25], Japan[10,14,18,28], Costa Rica[9], Italy[3], and Korea[4], in buffaloes in Pakistan[17], and in draught animals in Cambodia[15], Indonesia[1], Brazil[19] and Zambia (manuscript in preparation). However, there is little knowledge regarding BIV and bovine leukemia virus

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(BLV) infections in cattle in Turkey. BIV seropositivity has been associated with a decrease in milk production in dairy cattle[13], but has not been directly linked with clinical diseases in naturally infected cattle. In many cases, such a demonstration is complicated by the presence of confounding factors including co-infection with BLV. BLV is an oncogenic retrovirus that can cause lymphoid tumors and persistent lymphocytosis in its host though most infected cattle remain clinically and hematologically normal[2]. Many molecular aspects of BIV have been examined[7,8], but relatively little is known about the *in vivo* pathogenicity. This short communication provides initial data on the seroprevalence of BIV and BLV in Turkish cattle. In addition, BIV proviral DNA corresponding to a part of the *pol* gene from BIV-seropositive cattle was also detected.

Blood samples were collected from a total of 300cattle in four different cities, Balikesir, Tekirdag, Bursa and Canakkale in Turkey (Table 1 and Fig. 1). All animals which were older than one year were clinically normal. All cattle were of the Holstein breed, except 6 Simental cattle in Canakkale were also included. Blood samples (0.04ml) were absorbed on filter paper of strip type or type I (5 mm x 30mm) and dried according to the manufacturer's instructions (Toyo Roshi, Ltd, Tokyo, Japan). A section of the filter paper with blood was cut with scissors into 3-4parts, and soaked into 0.4ml of phosphate-buffered saline (PBS, pH7.2, 1 : 10 dilution) and then incubated at 4℃ overnight.

Anti-BIV and -BLV antibodies were detected by Western blot analysis (WBA) using the BIV *gag* protein, p26[14,15,17-19], and an immunodiffusion test using the BLV glycoprotein (gp 51) antigen as described by Onuma et al.[24], respectively.

To confirm BIV infection, blood samples from five seropositive cattle were collected with EDTA or heparin from BIV-seropositive animals. Peripheral blood mononuclear cells (PBMCs) and red blood cells were separated by centrifugation. DNA was extracted from PBMCs by the phenol-chloroform method and BIV proviral DNA was detected by nested polymerase chain reaction (PCR) as described below. The first amplification was done using a pair of outer primers specific to the BIV *pol* region (nt 2129-2148 : 5'-GTATCAGGCTCT TAAGGAAA-3', and nt 2541-2522 : 5'-TA ATCTTCTGGGTGGTAGTC-3'). The second amplification was performed to amplify a 298-bp fragment, using a pair of inner primers for the *pol* region (nt 2181-2220 : 5'-TCC GAAGCTGCTTGGGATAA-3', and nt 2479-2460 : 5'-TTCCACTGGAACCTCTCTAT-3') in the BIV genome[7,14,15,17-19,28] as described earlier[14,18,28]. The amplified products were fractionated on a 1.5% agarose gel, and visual-

Table 1. Detection of anti-BIV and anti-BLV antibodies in filter paper-absorbed blood of cattle in Turkey

City	NO. cattle tested	NO.BIV-seropositive (%)	NO. BLV-seropositive (%)
Balikesir	50	6 (12.0)	0 (0.0)
Tekirdag	41	4 (9.7)	0 (0.0)
Bursa	151	20(13.2)	5 (3.3)
Canakkale	58	7 (12.0)	0 (0.0)
Total	300	37(12.3)	5 (1.6)

-The tested cattle were older than one year and all of the Holstein breed, except 6 cattle of the Simental breed in Canakkale.
-Seroprevalence of BIV and BLV was tested by WBA and immunodiffusion test, respectively.

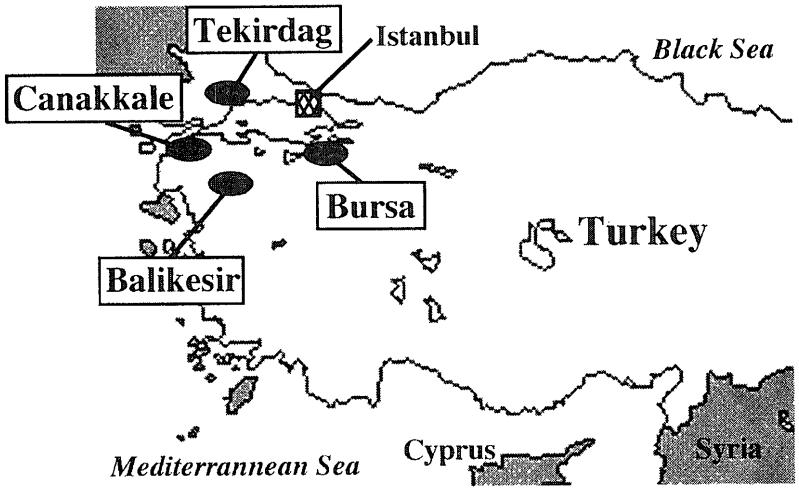

Fig. 1. Geographical positions of the areas in which blood samples were collected. Samples were collected from a total of 300 cattle in 4 different cities, Balikesir, Tekirdag, Bursa and Canakkale in Turkey.

ized by staining with ethidium bromide.

As summarized in Table 1, the prevalence of BIV in dairy cattle in each city ranged from 9.7 to 13.2% (average 12.3%), whereas that of BLV ranged from 0.0 to 3.3% (average 1.6%). Moreover, BIV-specific 298-bp fragments corresponding to part of the *pol* region were detected in five samples (data not shown). Since amplified products resulting from the PCR had been previously confirmed as BIV-specific by Southern hybridization and sequence analysis[14,15,18], confirmation was not conducted in the present study.

This is the first report of serological and molecular evidence for BIV infection in Turkish cattle. WBA and immunofluorescence were found to be equally sensitive for detecting BIV-seropositive animals.[30] However, Horzinek et al.[12] reported that immunofluorescence may give false positive results. On the other hand, Onuma et al.[21] has evaluated the syncytial assay for BIV detection and virus isolation using cell-culture systems. Techniques for the isolation of BIV are difficult[14] and expensive. Due to potential interference from other viral agents, especially bovine syncytial virus, a molecular analysis is required to specifically identify any agent cultured by this method. However, different PCR tests to detect BIV in cell culture, and experimentally and naturally infected cattle have been compared[27]. Consequently, we used two methods: WBA performed with a purified BIV *gag*, p26 protein, and nested PCR which was performed to detect BIV proviral DNA, a portion of the *pol* gene. Filter paper-absorbed blood of cattle was used to detect anti-BIV or -BLV antibodies in this study and the results were the same as those obtained with fresh sera (data not shown). In order to confirm BIV infection in cattle, PCR was performed, and part of the *pol* gene was amplified from DNA samples from different geographic locations[14,15,17,19,28]. However, parts of the surface envelope (SU) genes were not amplified by PCR from the same DNA sources of BIV-infected Turkish cattle, possibly due to the divergence in the nucleotide sequences of the SU gene[27], low copy number of the BIV genome, or disease stage of BIV-infected cattle (Suarez DL, personal communication). It has been shown that several distinct strains of BIV may exist

worldwide[14-17,19]. More recently, Meas et al.[16] reported that the nucleotide sequences of the *env* genes of all Japanese, Pakistan and Cambodian BIV field isolates were shorter, and several base substitutions were observed in the V1 region, and deletions were also found in the V2 region when compared to the American BIV isolates (R29, Florida 112, and Oklahoma 40) though these parts of Turkish BIV isolates still remain unknown.

Data from this study demonstrated that BIV infections were more common than BLV infections in Turkish cattle populations and the prevalence of BIV was not as high as in other countries in our previous studies[14-19,28]. These results also support the conclusion that infection with BIV and BLV can occur independently (Table 1) which is consistent with the other studies[4,14-19]. Indeed, BIV seropositivity had no correlation with BLV infection in this study. However, BIV infection was associated with a wide-range of clinical features that including weight loss, nerve degeneration, mouth ulcers and respiratory infections as the first case of bovine AIDS in the United Kingdom[5].

The primary target cell for BIV is generally regarded as the macrophage or precursor monocyte[22]. Onuma et al.[22] have shown that BIV infection in cattle reduces the responsiveness of various important monocytes without a change in CD4/CD8 ratios ; there was also a slight delay in the humoral antibody response to mouse serum proteins. Additionally, a new Florida BIV isolate (FL112) demonstrated no remarkable depletion of $CD4^+$ cells[30], unlike classical immunodeficiency viruses such as human immunodeficiency virus and feline immunodeficiency virus. However, B-cell proliferation was observed in calves inoculated with BIV FL 112 in short-term studies[30] and further investigation of the effects of the Turkish BIV isolates on acquired immune responses in cattle is warranted.

Although the route and mechanism of the natural transmission of BIV in cattle are largely unknown, our previous work has shown that BIV can be transmitted to offspring *in utero* or transplacentally as well as through colostrum or milk if dams are co-infected with BIV and BLV under natural conditions[18,23]. In contrast, another study has reported that BIV transmission is predominantly horizontal, rather than vertical, and may be environmentally influenced, perhaps by blood sucking insects[26]. BIV and BLV infections may have occurred via either vertical or horizontal transmission in Bursa, where there are lots of cattle in the herds (Table 1). However, the mode of BIV transmission in cattle in other areas in Turkey remains unknown as the movement of animals is poorly controlled (Table 1). A larger serological study with detailed long-term epidemiological observation will be necessary to confirm these preliminary findings and the role of BIV in disease progression in cattle has to be elucidated.

In summary, this epidemiological survey provides additional evidence that BIV and BLV infections are widespread in some cattle herds in Turkey and primary BIV infection in cattle may cause problems in animal health as reported in other countries around the world. The finding that a substantial proportion of cattle in Turkey were infected with BIV indicates that further investigation of the significance of this virus to cattle health is required.

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