Infection of Bovine Immunodeficiency Virus and Bovine Leukemia Virus in Water Buffalo and Cattle Populations in Pakistan

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ABSTRACT. A survey of antibodies to bovine immunodeficiency virus (BIV) known as bovine lentivirus and bovine leukemia virus (BLV) was conducted with samples from water buffalo and cattle populations in Pakistan. A total of 370 water buffaloes and 76 cattle were tested, and 10.3% and 15.8%, respectively, were found positive for anti-BIV p26 antibodies determined by Western blotting, while 0.8% of water buffaloes and no cattle were positive for anti-BLV antibodies determined by immunodiffusion test. BIV-seropositive water buffaloes and cattle were found to have BIV proviral DNA in the peripheral blood mononuclear cells determined by nested polymerase chain reaction. This is the first report of BIV infections in water buffaloes.—KEY WORDS: bovine immunodeficiency virus, bovine leukemia virus, water buffalo.

Bovine immunodeficiency virus (BIV), also known as bovine lentivirus is closely related to the human immunodeficiency virus type 1 (HIV-1) [8, 9]. Although BIV was originally isolated from an 8-year-old dairy cow with persistent lymphocytosis, progressive weakness, and emaciation and was designated as R29 in 1972 [26], it was only recently that additional isolations have been made [13, 23].

Serological evidence of BIV infection has been reported in many countries around the world [1–5, 7, 10–15, 20, 22], but the role of BIV infection in bovine disease progression remains unclear. BIV infection has been suspected to be associated with progression of bovine leukemia virus (BLV) [1, 9]. The occurrence of this infection was associated with secondary bacterial infections, stresses of parturition and early lactation and/or with unusual environmental stress cofactors, as reported in dairy herds [21]. BIV infection is also associated with decreased milk production shown by a large seroepidemiological study of dairy cows in Ontario, Canada [14]. In any event, bovine susceptibility to BIV infection may depend upon BIV strains, bovine breeds and environmental factors.

The purpose of current study is to determine whether Pakistan, water buffaloes and cattle were infected with BIV and/or BLV. Samples were collected from 370 water buffaloes and 76 cattle in Pakistan. Blood samples were bled with EDTA or heparin. Plasma samples were separated by centrifugation, mixed with β-propiolacton (final conc.0.4%) to inactivate live pathogens, and transported to our laboratory. All plasma samples were stored at -20°C until further use.

Antibodies against BIV-specific p26 protein were determined by Western blot analysis using the antigen prepared as described previously [13]. As summarized in Table 1, 10.3% of buffaloes and 15.8% of cattle tested were found seropositive for BIV infection. To demonstrate the correlation between BIV and BLV infections, these samples were also analyzed for anti-BLV antibodies by immunodiffusion test using the BLV glycoprotein antigen as described by Onuma et al. [19]. Few animals were found seropositive for BLV (0.8% in buffaloes, 0% in cattle). Only one water buffalo was found positive for both BIV and BLV infections, suggesting that the rate of the dual infection is very low in Pakistan.

In order to detect proviral DNA in infected animals, 25 BIV-seropositive buffaloes and cattle were randomly selected, and nested polymerase chain reaction (PCR) was performed using DNA samples prepared from their blood, as described previously [13]. The pol sequences are highly conserved among lentiviruses [13, 23–25], and BIV-specific 298-bp fragment corresponding to a part of the pol region was amplified from DNA samples of seropositive water buffaloes and cattle (data not shown). To further confirm the presence of BIV provirus, amplification of the V2 region of the surface envelope gene was also carried out using DNA samples from BIV-seropositive water buffaloes by nested PCR as described previously [24, 25]. As shown in Fig. 1, BIV-specific 432-bp fragment corresponding to the V2 region of the env was detected in all 25 samples examined as well as a positive control, BIV strain R29. Thus, BIV proviruses were indeed present in peripheral blood mononuclear cells of seropositive animals.

Since sequence variations have been reported in the
envelope gene between different BIV strains, nucleotide sequence analysis of the V2 regions is now in progress for molecular epidemiology of BIV. This is the first report of the serological and proviral detection of BIV in water buffaloes. This seroepidemiologic survey provides evidence that BIV infection is widespread around the world, and that not only dairy and beef cattle, but also water buffaloes were infected. However, the occurrence of natural transmission of BIV is unknown, BIV proviral DNA had been found in milk [13, 16]. Whether BIV is transmitted via uterus, placenta, colostrum, or milk is under investigation. Proviral DNA of BIV was also detected in bull semen [17], and the seroprevalence of BIV infection increases according to 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 [22]. Moreover, pathogenesis of this virus still remains unclear although BIV infections can induce dysfunction of monocytes [18], neutrophils [6] and BIV proviral DNA was also detected in various organs from the cow naturally infected with BIV by nested-PCR systems targeting the pol region [13]. In conclusion, this study can provide additional information about contribution of BIV infection in various countries around the world.

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Table 1. Seroprevalence of BIV and BLV in water buffalo and cattle populations in Pakistan

<table>
<thead>
<tr>
<th>Animals</th>
<th>No. of tested animals</th>
<th>No. of BIV-seropositive animals (%)</th>
<th>No. of BLV-seropositive animals (%)</th>
<th>No. of dual infection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffalo</td>
<td>370</td>
<td>38 (10.3)</td>
<td>3 (0.8)</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>Cattle</td>
<td>76</td>
<td>12 (15.8)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>

a) Seroprevalence of BIV and BLV was detected by Western blotting and immunodiffusion tests, respectively.

Fig. 1. PCR amplification of the V2 region of the surface envelope gene [24] of BIV proviral DNA in BIV-seropositive water buffaloes (Lanes 1–9) compared with positive control (lane P, a DNA sample obtained from BIV R29-infected bovine embryo spleen cells) and negative control (Lane N, a DNA sample prepared from BIV-seronegative cow [13]). Lane M: size marker.

REFERENCES