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## Evidence for bovine immunodeficiency virus infection in cattle in Zambia

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### Abstract

We report herein on the first evidence for the presence of bovine immunodeficiency virus (BIV) in Zambia. Serological surveillance of BIV and bovine leukemia virus (BLV) was conducted in traditional cattle herds in Zambia. Out of a total of 262 sera analyzed, 11.4% were found positive for anti-BIV p26 antibodies as determined by Western blot analysis, while 5.0% were positive for anti-BLV gp51 antibodies as detected by immunodiffusion test. Peripheral blood mononuclear cells from BIV seropositive cattle were found to have BIV-provirus DNA, as detected by nested polymerase chain reaction. A nucleotide sequence corresponding to a 298 bp fragment of the BIV *pol* gene was also analyzed. Amino acid sequences of these Zambian *pol* gene products showed 98.0 to 100% homology to the American strain BIV R29, 97.0 to 99.0% to Japanese BIV isolates, and divergence ranged from 0.0 to 2.0% among Zambian BIV isolates.

KEY WORDS : bovine immunodeficiency virus ; bovine leukemia virus ;  
molecular evidence ; seroprevalence ; Zambia.

### Introduction

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 in

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the United States of America (USA). The cow demonstrated persistent lymphocytosis, progressive weakness and emaciation. Necropsy revealed the presence of clinical and histopathological changes, including mild lymphocytic perivascular cuffing in brain<sup>22</sup>. Studies have shown that BIV resembles human immunodeficiency virus (HIV) type 1 and simian immunodeficiency virus in its structural, genetic, antigenic and biological properties<sup>3,4</sup>. However, conclusive evidence that BIV causes immunodeficiency in cattle has not been established.

Since BIV recognition as a lentivirus in the late 1980s<sup>3,4</sup>, BIV infection has been shown to be prevalent globally and the results of these surveys are dependent upon the choice of antigen used in the detection assay and the degree of cross-reactivity between the antigens and the circulation of the field-isolated virus strains. Only one epidemiological study has shown that BIV infection associates with decreased milk production in cows<sup>7</sup>, but the disease has not been directly linked with clinical disease in naturally infected cattle. BIV infection in cattle has been suspected to be associated with a wide-range of clinical spectra that include weight loss, nerve degeneration, oral ulcers, respiratory infections as was observed in the first case of bovine acquired immunodeficiency syndrome (AIDS) in the United Kingdom<sup>2</sup>, and with incidence of protozoan diseases<sup>9</sup> and tuberculosis<sup>17</sup>. In some instances, such a manifestation is complicated by the presence of confounding factors including co-infection with bovine leukemia virus (BLV) in cattle industry<sup>8,19,21</sup>. BLV is an oncogenic retrovirus that can cause lymphoid tumors and persistent lymphocytosis in its host with most infected cattle remaining clinically and hematologically normal<sup>1</sup>.

In Zambia, the prevalence status of BIV and BLV in cattle is currently unknown. The

purpose of this study was to gather initial data on the seroprevalence of BIV and BLV in Zambian cattle and in addition the nucleotide sequence of the *pol* genes of BIV proviral DNA from BIV seropositive cattle was also analyzed.

## Materials and Methods

### *Blood, Plasma and DNA samples*

Blood samples were collected with EDTA or heparin from a total of 208 cattle in Mumbwa district, central province in early 2000 and 54 cattle in Lusaka province at the end of 2001, respectively. All animals were clinically normal and older than one year at the start of this study. Plasma, peripheral blood mononuclear cells (PBMCs) and red blood cells were separated by centrifugation. All plasma samples were mixed with  $\beta$ -propiolacton (final conc., 0.4%) to inactivate live pathogens. DNA was extracted from PBMCs by SepaGene kit according to the manufacturer's instruction (Sanko Junyaku Co., Ltd., Tokyo, Japan) and was then transported to our laboratory. All plasma and DNA samples were stored at -20 and -80 °C, respectively, until further use.

### *Western blot analysis (WBA)*

In order to detect antibodies against BIV protein, WBA was performed using culture supernatant fluid of bovine embryonic spleen cells infected with BIV R29 as an antigen. This BIV p26 *gag* antigen was prepared as described previously<sup>8,9,11,21</sup>.

### *Immunodiffusion test (IDT)*

For the detection of anti-BLV antibodies, the IDT using the glycoprotein antigen prepared from culture supernatant fluid of BLV-infected fetal lamb kidney cell lines. This BLV gp51 antigen was prepared as described by Onuma et al<sup>16</sup>.

### *Polymerase chain reaction (PCR)*

To detect the BIV proviral DNA, nested PCR was performed to detect a portion of the *pol* gene. A BIV-specific band with the predicted size of 298 bp was detected in each of the DNA samples tested with primer sets as described earlier<sup>8,9,13,21</sup>. Each PCR was done in a buffer containing 45 mM Tris-HCl (pH 8.8), 11 mM ammonium sulfate, 4.5 mM MgCl<sub>2</sub>, 6.7 mM 2-mercaptoethanol, 4.4 mM EDTA (pH 8.0), 113 (Mg/ml BSA, 1 mM dNTPs, 20 pM each of the oligonucleotide primers and 2.5 U of *Taq* DNA polymerase (Takara Shuzo Co., Ltd., Kyoto, Japan). The PCR was carried out in an automatic DNA thermal cycler (Perkin Elmer-Applied terms, Foster City, USA). Cycling conditions for the first step as well as second step PCR were 94 °C for 3 min, followed by 35 cycles of 94 °C for 30 sec, 57 °C for 45 sec and 72 °C for 30 sec, with a final extension step at 72 °C for 7 min. The PCR products were separated in a 1.5% agarose gel with ethidium bromide and electrophoresed at 100 V for 35 min. The reaction was determined to be positive or negative based on product of the appropriate size being visible on UV exposure.

### *DNA cloning and sequencing*

PCR products of *pol* gene of Zambian BIV field isolates were excised from the gel and purified with the GeneClean II kit (Bio 101, La Jolla, USA), and cloned into pGEM-T easy vector (Promega, Madison, USA). The pGEM-T plasmids containing the inserts corresponding to these *pol* regions of BIV field isolates were purified by standard mini-prep method, and sequenced using the BigDye terminator sequencing kit (Perkin Elmer-Applied terms, Foster City, USA) and Model 310 genetic analyzer (Perkin Elmer-Applied terms, Foster City, USA). At least 4 individual plasmid clones per each BIV isolate were used

for sequencing. DNA sequence analysis was done using the GENETYXMAC 10.1.2 package (Software Development Co., Ltd., Tokyo, Japan) in combination with the BLAST program at the GenBank for homology search and comparison with known BIV gene sequences.

## **Results**

### *Seroprevalences of BIV and BLV in Zambian cattle*

Antibodies against BIV p26 protein were detected in Zambian plasma samples by WBA and the same samples were also tested for anti-BLV antibodies by IDT. As summarized in Table 1, among tested cattle, 11.4% of cattle were found to have BIV seropositive, while 5.0% of cattle were BLV seropositive.

Table 1. Seroprevalence of BIV and BLV in cattle in Zambia<sup>a</sup>

No. of tested cattle	No. of seropositive cattle (%)	
	BIV	BLV
262	30 (11.4)	13 (5.0)

a) Seroprevalence of BIV and BLV was determined by Western blotting and immunodiffusion test, respectively.

### *Molecular characterization of Zambian BIV isolates within *pol* genes*

In order to further confirm BIV seropositivity in Zambian cattle, PBMCs from BIV seropositive animals were found to have BIV-provirus DNA using nested PCR to detect a 298 bp fragment of the *pol* gene (nt2181-2479). By using the BLAST program, all nucleotide sequences were shown to be highly homologous to the respective sequences of a molecular clone, BIV R29 and other known BIV sequences in the database. As shown in Table 2, nucleotide sequence of Zambian BIV isolates (3, 6, 11 and 17) showed 99.0 to 99.3% homology with the American BIV strain R29, and 98.0 to 98.7% with Japanese BIV isolates and 99.0 to 100% similarities to a Brazilian

Table 2. Comparison of nucleotide and amino acid sequences of *pol* gene of Zambian, Japanese and Brazilian BIV isolates<sup>a)</sup>

	R29	JpnKa 3	BrazilC 7	Zamb 3	Zamb 6	Zamb11	Zamb17
R29		98.0	99.0	99.3	99.3	99.3	99.0
JpnKa 3	98.0		98.0	98.7	98.0	98.7	98.0
BrazilC 7	100	98.0		99.7	99.7	99.7	99.7
Zamb 3	99.0	99.0	99.0		99.3	99.3	99.3
Zamb 6	100	98.0	100	99.0		99.3	99.3
Zamb11	99.0	99.0	99.0	100	99.0		99.3
Zamb17	100	98.0	100	99.0	100.0	99.0	

a) Percentage nucleotide (upper half) and amino acid (lower half) sequences similarities of the *pol* gene (nt 2181-2479) of the predicted size of 298 bp-99 aa among Zambian (Kasisi farm [Boran breed, beef cattle], Lusaka province), Brazilian (Campo Grande area), Japanese (Kamikawa district) and American R29 BIV isolates. Nucleotide sequence of BIV strain R29 (molecular clone R29-127) was obtained from the GenBank.

BIV isolate<sup>13)</sup> and each other. Deduced amino acid sequences were highly homologous to all Zambian BIV field isolates within *pol* genes with American, Japanese and Brazilian BIV isolates (Table 2) and molecular characterization of Zambian BIV isolates within *env* gene still remains unknown.

### Discussion

Although the number of tested animals was limited, this is the first report of serological and molecular evidence for BIV infection in cattle in Zambia. BIV seropositivity was 11.4% of the total tested animals. The common serological diagnostic test for BIV has been widely used for assessing the prevalence of BIV. However, loss of antibody to BIV p26 *gag* antigen in experimentally infected cattle has been reported<sup>5)</sup>. Thus, the use of a test that directly identifies the BIV genome is recommended to positively identify infected animals. Sequence homologies among BIV isolates have been observed to be much higher in the *pol* region than in the surface envelope genes (*env*) region<sup>10)</sup> and as such, *pol* primers would more likely identify more field isolates than the *env* PCR test<sup>20)</sup>. In this study, the combination of WBA and *pol* region specific PCR tests was used for the identification of

BIV-infected animals, and the *pol* region was detected from all 30 seropositive cattle whereas the *env* region primers were unable to amplify any fragment.

The present study shows that BIV is more common than BLV in Zambian cattle populations, however, the prevalence rate of BIV was not so high as compared to other countries reported in previous studies<sup>8,9,11,13,21)</sup>. Moreover, these results support the conclusion that infection with BIV and BLV can occur independently. Indeed, BIV seropositivity has no correlation with BLV infection and no co-infection was observed in this study (Table 1). However, BIV infection in cattle has been associated with secondary bacterial infections, stresses of parturition and early lactation and/or with unusual environmental stress in a Louisiana dairy cattle herd<sup>18)</sup>. BIV infection in cattle in Zambia may associate with common bacteria such as *E. coli* and *Salmonella* spp. that have been isolated from aborted fetuses and semen samples in Zambian cattle<sup>14)</sup> and may also co-infect with bovine viral diarrhoea virus or infectious bovine rhinotracheitis virus or/and *Brucella*<sup>6)</sup>.

Effective control of diseases cannot be achieved until the modes of transmission are known. Most lentiviruses are in general

spread both horizontally and vertically by exchange of body fluids. Although the route and detailed mechanism of BIV natural transmission in cattle is largely unknown, our previous work has shown that BIV can be transmitted to offspring *in utero* or transplacentally and BLV can be transmitted through colostrum or milk if dams are co-infected with both BIV and BLV under natural conditions<sup>12,15</sup>. Moreover, it had been indicated that horizontal transmission, possibly mediated by biting insect vectors, might be a significant factor in the spread of BIV in warmer regions of the southern USA<sup>19</sup>. Both modes of transmissions may be suspected for BIV in cattle in Zambia and other parts of southern tropical Africa as have been observed in tropical and subtropical Asia<sup>8,9,11,13,21</sup>. To confirm these preliminary findings, a larger serological study with detailed long term epidemiological observation will be necessary and the role of BIV in diseases progression in cattle has to be elucidated.

In conclusion, this epidemiological survey provides additional evidence that BIV and BLV infections are widespread in some cattle herds in Zambia. The finding that a substantial proportion of tested cattle in Zambia were infected with BIV indicates that further investigation of the significance of this virus to cattle health is required.

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