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# Cloning of the Bovine Immunodeficiency Virus *gag* Gene and Development of a Recombinant-Protein-Based Enzyme-Linked Immunosorbent Assay†

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**An enzyme-linked immunosorbent assay (ELISA) was established for the rapid detection of specific bovine immunodeficiency virus (BIV) antibodies in cattle, using recombinant Gag protein as an antigen. The *gag* coding region from BIV was cloned into an expression vector, pQE32, which expressed high levels of recombinant protein from *Escherichia coli*. The ELISA was standardized by a checkerboard titration against known BIV-positive and -negative sera from cattle and a monoclonal antibody to the Gag protein. A total of 139 cattle serum samples, from the diagnostic laboratory at Kansas State University, Manhattan, and from the Dairy Station, Louisiana State University, Baton Rouge, were compared by ELISA and immunoblot assays for the detection of BIV-specific antibodies. Of 26 cattle sera samples which tested positive using the immunoblot assay, 23 were positive by ELISA, thus establishing a strong correlation between the two tests. The sensitivity and specificity of ELISA relative to immunoblotting were 0.88 and 0.93, respectively. ELISA proved to be as specific as immunoblotting but was much less time-consuming and easier to perform.**

Bovine immunodeficiency virus (BIV) is a lentivirus that causes lymphadenopathy, lymphocytosis, central nervous system lesions, progressive weakness, and emaciation (20, 21, 24). Studies have shown that BIV resembles human immunodeficiency virus (HIV) type 1 and other lentiviruses, e.g., equine infectious anemia virus and feline immunodeficiency virus, in its structural, genetic, antigenic, and biological properties (5, 6, 16). Although BIV has been characterized in detail molecularly (5, 6, 7, 11, 15), little is known about the biology of naturally occurring BIV infection. There has been no confirmed evidence of association of BIV infection with a naturally occurring disease condition. However, BIV infection was reported to be associated with decreased lymphocytic blastogenic response (12) and milk production (13). A possible immune dysfunction in BIV-infected calves, with a drop in CD4/CD8 ratio and a decrease in antibody response to virus vaccines, was also reported (27). Progress in the development of rapid tests for diagnosis of BIV in cattle has been slow because of the inability to generate adequate amounts of BIV antigens required for seroepidemiological studies. BIV can be propagated well only in primary bovine cell cultures, and there is a lack of availability of continuous cell lines expressing high levels of BIV. The yields of native viral protein from primary bovine cell culture are quite low (24), and those proteins show a high background reactivity with negative control sera in serological tests (25). Thus, there is a need for recombinant BIV antigens to facilitate studying the epidemiology and prevalence of BIV antibodies.

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The BIV genome has been cloned, and the complete nucleotide sequence has been determined (5). Among the structural proteins predicted by the nucleotide and amino acid sequences of BIV is the core protein encoded by the *gag* gene. The Gag precursor of BIV has been shown to have a molecular mass of 53 kDa (17), and, by analogy to HIV cleavage products, it is predicted to be processed into three smaller proteins, p17, p26, and p15 (10, 14), which are the matrix, capsid, and nucleocapsid proteins, respectively. Immune sera from calves experimentally infected with purified BIV detected p26 proteins in immunoblot analysis (25). This suggested that p26 is the major core, or capsid, protein of BIV. Thus, the *gag* gene product is an important viral antigen that induces a strong immune response in infected cattle. Recently, a purified recombinant BIV Gag protein was used in an immunoblot assay to detect BIV antibodies in field bovine serum samples (29). The method proved to be sensitive and specific by comparing the results with PCR (29). However, the immunoblotting is tedious and time-consuming. In addition, the recombinant Gag protein was expressed in pATH10 system as a TrpE fusion protein (3). The TrpE fusion protein accounts for more than 50% of the recombinant protein. Since calves are exposed to a variety of bacterial infections, the large proportion of fusion protein gives high background reactivity. Therefore, the fusion proteins may not be suitable to be used as an enzyme-linked immunosorbent assay (ELISA) antigen. Furthermore, the expressed level of Gag fusion protein in the pATH10 is relatively low, and it is difficult to purify required amounts of the specific protein. The present study was undertaken to express recombinant Gag protein in higher quantities and to develop an ELISA for the diagnosis of BIV infection.

## MATERIALS AND METHODS

**Construction of prokaryotic BIV expression plasmids.** For expression of BIV in *Escherichia coli*, the pATH *gag*-3 clone, containing 781 bp of the *gag* coding

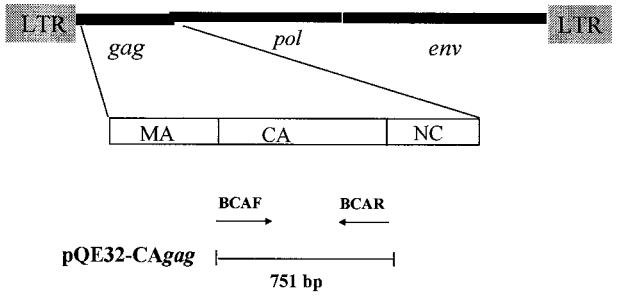


FIG. 1. BIV *gag* construct. The thick horizontal bars represent the relative genomic organizations of the *gag*, *pol*, and *env* coding regions of BIV. The large open box represents the unprocessed $p53^{gag}$ precursor protein. The Gag precursor is processed into three smaller proteins: matrix (MA), capsid (CA), and nucleocapsid (NC). Only the 751-bp capsid region was cloned into pQE32 vector. BCAF, BIV capsid forward primer; BCAR, BIV capsid reverse primer; LTR, long terminal repeat.

region, was used as a PCR template to generate a 751-bp *gag* product (Fig. 1). The 5′ primer (GGATCCAGGCCAGAGCTGATAAGGAA) corresponded to nucleotides (nt) 644 to 664 in the BIV genome, with a *Bam*HI site added at the beginning (underlined). The 3′ primer, AAGCTTATCCCACTACCCTACATG CT, corresponded to nt 1375 to 1395 in the BIV genome, with a *Hin*dIII site added (underlined). PCR amplification with these primers left the *Bam*HI and *Hin*dIII restriction sites at the ends of the product. pQE32, a modified version of pDS56RBSII, a prokaryotic expression vector that uses the T5 promoter and two *lac* operator sequences, was used to express the BIV *gag* cDNA. The pQE32 vector was replicated in a bacterial strain, *E. coli* M15, which expresses a high level of the cloned gene under control of the *lac* repressor, permitting induction of recombinant protein expression by isopropyl-β-D-thiogalactoside (IPTG). pQE32-*gag* was constructed by ligating the PCR product into a T vector by a standard ligation procedure (19). Subsequently, the PCR product, digested with *Bam*HI and *Hin*dIII, was cloned into pQE32 using the same enzymes. The two restriction sites enabled the cloning of the *gag* cDNA in the correct orientation, ensuring translation in frame. The *gag* cDNA sequence at the junction region in pQE32-*gag* was determined by the dideoxyribonucleotide chain termination method (19).

**Expression and purification of recombinant Gag protein.** The construct was maintained in *E. coli* M15, and expression of the Gag protein was induced with 2 mM IPTG for 4 h. *E. coli* strain M15 transformed with a pQE32 expression vector containing the BIV *gag* gene, or transformed with plasmid pQE32 alone as a negative control, was grown in 1.5 ml of Luria-Bertani broth containing both ampicillin and kanamycin overnight at 37°C. The cultures were then inoculated with fresh Luria-Bertani broth (1:4 dilution) and incubated for 30 min at 37°C. IPTG was added to a final concentration of 2 mM to induce protein expression. After 4 h of induction, cells were harvested by centrifugation at 4,000 × *g* for 15 min and lysed by sonication in lysis buffer B (8 M urea, 0.1 M $NaH_2PO_4$, 0.01 M, and Tris-HCl, pH 8.0). The recombinant Gag proteins were analyzed by sodium dodecyl sulfate–12.5% polyacrylamide gel electrophoresis (SDS–12.5% PAGE) and purified by using Ni-nitrilotriacetic acid (Ni-NTA) columns (the polyhistidine tag at the amino terminus of the recombinant Gag protein binds to Ni-NTA resin) (Pierce, Rockford, Ill.). The purified-protein concentration was determined by a dye-binding protein assay (Pierce). The Gag protein was then aliquoted and stored at −20°C until use.

**Immunoblot assay.** Purified recombinant Gag protein was used as the antigen in the immunoblot assay. The protein was loaded onto a preparative SDS–12.5% PAGE minigel (Bio-Rad, Hercules, Calif.) and electrophoresed in Tris-glycine buffer (0.025 M Tris base, 0.192 M glycine, 0.1% SDS) at 30 mA/gel for 1 h. The protein was then transferred onto a nitrocellulose membrane (0.45-μm pore size) with a transblot apparatus (Bio-Rad) at 259 mA for 1 h. The membrane was blocked with 2% bovine serum albumin–0.02 M Tris base–0.385 M NaCl–0.1% Tween 20 (pH 7.5) (TTBS) at room temperature for 2 h, rinsed three times with TTBS, and then cut into 5-mm strips (each strip contained 0.1 μg protein). Unknown and control sera were diluted 1:50 with TTBS in multichannel incubation trays, and the strips were put into each channel and incubated overnight at 4°C. After being washed three times with TTBS, the strips were incubated with horseradish peroxidase-labeled goat anti-bovine immunoglobulin G (IgG) (heavy plus light chains) (1:3,000) (Kirkegaard & Perry Laboratories, Gaithersburg, Md.) for another 2 h at room temperature and rinsed twice with TTBS and once with TBS (0.02 M Tris base, 0.385 M NaCl, pH 7.5). Finally, the color was developed with a 4-CN substrate solution (100 ml of TBS, 60 μl of $H_2O_2$, 20 ml of ice-cold methanol, and 60 μg of 4-chloronaphthol [Pierce]) at room temperature for 15 min.

Sera from calves either naturally or experimentally infected with BIV and a monoclonal antibody to BIV Gag protein (27, 28) were included as positive controls in the experiment. Known calf sera negative for BIV and calf sera positive for either bovine viral diarrhea virus (BVDV) or bovine herpesvirus 1 were also included as negative controls in the experiment.

**ELISA.** Preliminary titration experiments were conducted to determine optimum reagent concentrations using specific monoclonal antibodies and known positive and negative control bovine sera. Immulon-I microtiter plates (Dynatech Laboratories, Chantilly, Va.) were coated with purified recombinant Gag protein (0.4 μg/well). The antigens were diluted in 0.05 M sodium carbonate buffer (pH 9.6), and a 100-μl volume was added to each well and incubated at 4°C overnight. After coating, the microtiter plates were washed with 0.01 M phosphate-buffered saline (PBS)–Tween 20 (0.05%) (PBST) buffer, blocked with 0.05% glycine in PBS (100 μl/well), and incubated at 37°C for 30 min. Test sera, diluted 1:50 in PBST, were added to three wells (100 μl/well) and incubated at 37°C for 30 min. Control sera were twofold serially diluted, from 1:50 to 1:200, and tested against the antigen. After five washes with PBST buffer, 100 μl of a 1:5,000 dilution of peroxidase-conjugated rabbit anti-bovine IgG or goat anti-mouse IgG (Kirkegaard & Perry Laboratories) in PBST was added to each well, and the plates were incubated at 37°C for 30 min. The plates were then washed with PBST buffer, followed by the addition of 100 μl of ABTS [2,2′-azinobis(3-ethylbenzthiazoline-sulfonic acid)] substrate (Kirkegaard & Perry Laboratories) to each well. The plates were incubated at 37°C for 30 min. The absorbance was measured at 410 nm, and the cutoff value was based on the average optical density (OD) of the negative control.

**PCR.** Sample DNA was extracted from the clotted bovine blood sample. Briefly, after removal of the serum, the clotted blood was vigorously washed with PBS, and blood cells released from the clot were collected. After centrifugation, the pelleted blood cells were suspended with DNA extraction buffer A (100 mM KCl, 10 mM Tris [pH 8.3], 2.5 mM $MgCl_2$) and then incubated with an equal volume of extraction buffer B (10 mM Tris [pH 8.3], 2.5 mM $MgCl_2$, 1% NP-40, 120 μg of proteinase K per ml) at 60°C for 1 h. DNA was then extracted with a phenol-chloroform solution, followed by precipitation with isopropanol. After washing with 70% alcohol and air drying, DNA was suspended in distilled water and the DNA concentration was determined. DNAs from bovine blood samples and BIV-positive and BIV-negative control DNAs were amplified by PCR using a pair of primers designed to target a 242-bp highly conserved BIV *pol* gene. Thermal cycling was performed using a 35-cycle profile of 95°C for 1 min, 50°C for 1 min, and 72°C for 1 min (29). The PCR products were analyzed by 1.2% agarose gel electrophoresis.

**Field samples.** Forty bovine serum samples with clotted blood (from Kansas) were submitted to the Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Kansas State University, during 1999. Most of the cattle were clinically normal, and the samples were submitted for routine serological tests. Another 99 serum samples were obtained from the Louisiana Agricultural Experiment Station, Baton Rouge Dairy Station.

**Statistical analysis.** A statistical analysis was performed on the data using the SAS system. McNemar's test was applied to the results that differed between the ELISA and immunoblot methods.

## RESULTS

**Cloning, sequencing, and expression in *E. coli* of BIV Gag protein.** One 751-bp *gag* gene, corresponding to nt 644 to 1395 of the BIV genome, was made by PCR amplification (Fig. 1) and subcloned into the prokaryotic expression vector pQE32, designated pQE32-*gag*, for the production of Gag protein. pQE32-*gag* was sequenced across the fusion junction to ensure preservation of the reading frame. The sequence results (data not shown) indicated that the recombinant 29-kDa Gag protein contains only 13 amino acids from the bacterial part, which accounts for 5% of the total protein. After electrophoresis and Coomassie blue staining, a distinct 29-kDa Gag-specific band was observed in the bacterial lysates containing pQE32-*gag* after IPTG induction (Fig. 2a, lane 2). The observed molecular mass was in agreement with the predicted molecular mass of 29 kDa from the construct. The protein was purified by using Ni-NTA columns and is shown as one band in SDS-PAGE (Fig. 2a, lane 3). The total cellular protein from a clone containing the plasmid, pQE32, served as the negative control (Fig. 2a, lane 1). The recombinant protein constituted approximately 25% of the total protein after IPTG induction. The final yield of the fusion protein was about 1,500 μg/ml of bacterial culture (data not shown).

**Immunoblot detection of Gag proteins.** In order to test the reactivities of the expressed Gag protein, immunoblot analysis was carried out using a monoclonal antibody against Gag pro-

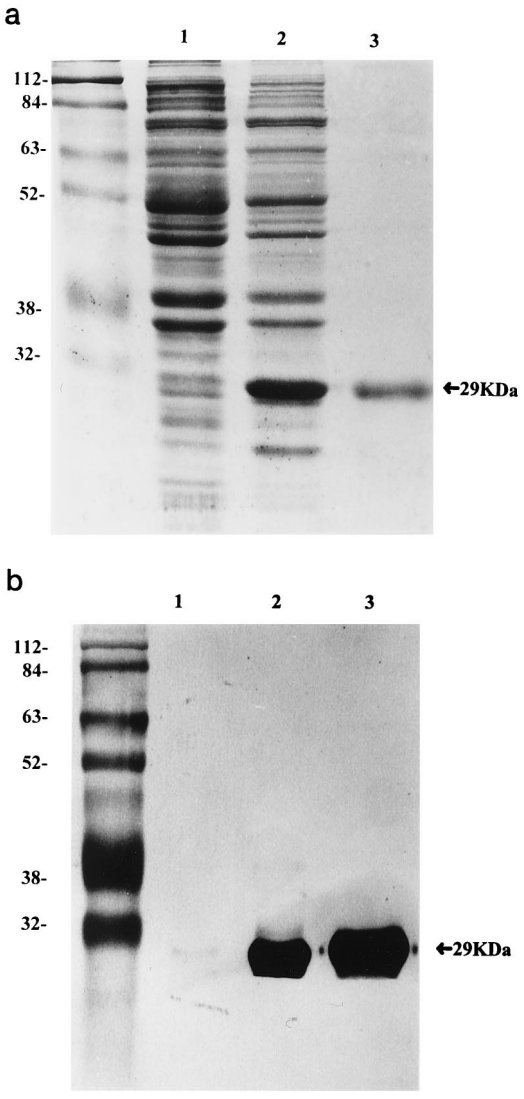


FIG. 2. SDS-PAGE with Coomassie blue staining and immunoblot analysis of pQE32-expressed Gag protein. Aliquots of induced proteins from pQE32 (lanes 1) and from pQE32-*gag* before (lanes 2) and after (lanes 3) purification were subjected to SDS–12.5% PAGE. (a) Coomassie blue-stained gel. The arrow indicates the 29-kDa Gag protein. (b) Immunoblot analysis of Gag protein. Molecular masses in kilodaltons are given for markers at the left.

tein and two known naturally infected and immunized sera. The immune serum was obtained from an animal immunized against BIV (27, 28). Production of monoclonal antibody against *gag* was described previously (27, 28). The monoclonal antibody specifically recognized a 29-kDa Gag protein in IPTG-induced, pQE32-*gag*-transformed bacterial extracts and purified Gag protein (Fig. 2b, lanes 2 and 3). The absence of any reactive band in the lysate containing pQE32 plasmid alone indicated that the reaction is specific for the BIV antigen (Fig. 2b, lane 1). During antigen titration, 0.2 μg of purified recombinant BIV Gag protein on 5-mm strips produced a sharp band with all of the antibody dilutions of BIV-positive sera. The BIV Gag protein showed a strong and specific reaction with sera collected from calves naturally and experimentally infected with BIV (Fig. 3, lanes 1 and 2). Normal serum and two known BIV-negative sera did not recognize the Gag protein (Fig. 3, lanes 3, 4, and 5). Control sera with antibodies to other bovine viruses, e.g., BVDV and bovine leukemia virus (BLV), did not react with the BIV Gag protein (Fig. 3, lanes 6 and 7). Representative BIV-positive samples (lanes 8 and 9) and BIV-negative samples (lanes 10 and 11) were also included. The minor band below the 29 kDa in lane 2 may result from the degradation product of Gag protein.

**Optimization of ELISA using recombinant Gag protein.** Preliminary titration experiments were conducted to determine optimum reagent concentrations and Immulon plates using specific monoclonal antibodies and known BIV-positive and -negative bovine sera. The most optimal and consistent results were obtained when 0.4 μg of Gag protein was used to coat each wall of the Immulon I plate. Sample concentrations were determined by standard checkerboard titration, using 0, 1:50, 1:100, and 1:200 dilutions, with the 1:50 dilution found to be optimal based on the best signal-to-noise ratio. Samples were run in triplicate; the greatest outlier OD reading was discarded, and the remaining two values were averaged to give an adjusted OD reading for each sample. This was done for each positive and negative control for each run. The greatest outlier OD reading being discarded was determined based on the coefficient of variation (CV). If the CV for the OD reading in each sample was greater than 5%, the OD reading was discarded. If all the three CVs for each sample were within 5%, the OD reading with the largest CV was discarded. Signal-to-positive ratios for each sample were determined according to the following formula used in the Kansas State University diagnostic laboratory (26a): (adjusted $OD_{sample}$ − adjusted $OD_{negative}$)/(adjusted $OD_{positive}$ − adjusted $OD_{negative}$).

In order to establish a positive cutoff point, the adjusted ODs of the negative controls were averaged and compared with the average adjusted ODs of all positive controls, according to the formula [(average adjusted $OD_{negative}$)/(average adjusted $OD_{positive}$ − average adjusted $OD_{negative}$)] × 2, which gave a cutoff of 0.76 with a 95% lower confidence limit. Samples were judged to be positive if the sample signal-to-positive ratio was equal or greater than 0.76. Using this cutoff value, the ELISA results for 139 samples were obtained. The comparison of the results with those obtained by immunoblotting are summarized in Table 1. A total of 139 samples from Kansas State University and Louisiana State University were tested by both ELISA and immunoblotting. The number of positive samples as determined by both tests was 23, and the number of negative samples by both tests was 105. Three samples were positive by immunoblotting but negative by ELISA, while eight samples positive by ELISA were negative by immunoblotting. All BIV-positive samples by immunoblotting were confirmed by PCR (data not shown). Using standard formulas (23) to determine sensitivity and specificity, immunoblot results were treated as the true status. Sensitivity and specificity using immunoblot as the true status can be calculated according to the following equations: sensitivity = $A/(A + C)$ and specificity = $D/(B + D)$, where $A$ is the number of samples ELISA positive and immunoblot positive, $B$ is the number ELISA positive and immunoblot negative, $C$ is the number ELISA negative and immunoblot positive, and $D$ is the number ELISA negative and immunoblot negative. With values for $A$, $B$, $C$, and $D$ of 23, 8, 3, and 105, respectively, the sensitivity equals 88% and the specificity equals 93%. A statistical analysis was performed on the data sets using McNemar's method. McNemar's test asked whether the results of the ELISA and immunoblotting were different, and no significant difference was found at the $P$ level of 0.10. In conclusion, the two methods agreed with each other.

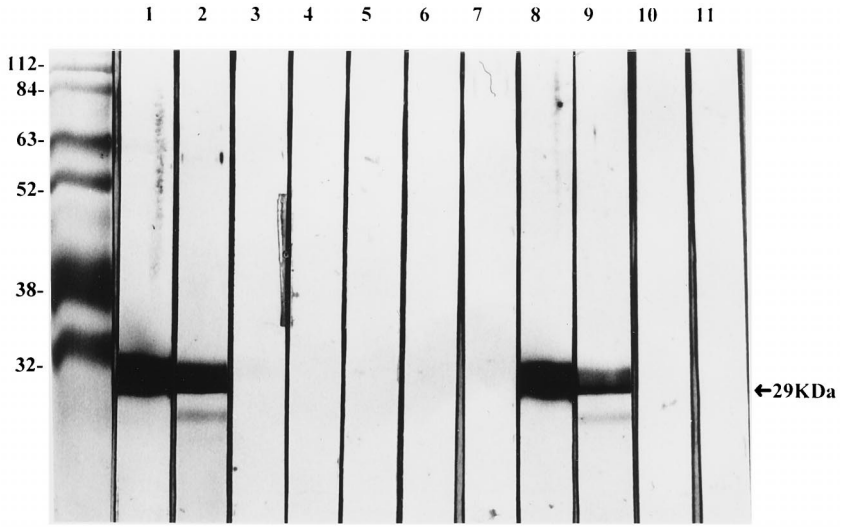


FIG. 3. Immunoblot analysis using BIV Gag protein as the antigen and probing with serum from cattle naturally infected with BIV (1:100) (lane 1), serum from cattle experimentally infected with BIV (1:100) (lane 2), sera from one normal calf and two non-BIV-infected calves (1:20) (lanes 3, 4, and 5), BVDV- and BLV-positive sera (1:20) (lanes 6 and 7), representative tested BIV-positive serum samples (1:100) (lanes 8 and 9), and tested BIV-negative serum samples (lanes 10 and 11). Molecular mass standards in kilodaltons are given for markers at the left. The arrow indicates a BIV-specific band.

## DISCUSSION

BIV infection is prevalent worldwide, and seropositive cattle have been identified in many countries (1, 4, 7, 8, 9, 13, 22). Although there were no reports on direct associations of BIV infection and naturally occurring disease among cattle, there were also no large-scale surveys on the prevalence and distribution of BIV among cattle. The lack of large surveys for BIV was due partly to the difficulty in obtaining large amounts of BIV antigen for the test. In order to develop a rapid diagnostic test for BIV antibodies in bovine serum, we cloned the p26 Gag protein from BIV strain R29 and developed an ELISA using the recombinant Gag protein as an antigen for detection of BIV antibody.

As a potential diagnostic tool, a recombinant-protein-based ELISA for BIV infection offers many advantages over other methods. Using recombinant Gag protein as an antigen for diagnosis of BIV will eliminate the use of native viral protein, which is difficult to produce. High levels of protein expression are attainable in a prokaryotic expression system contributing to the purity of the final antigen preparation, thus improving the specificity of the immunoassay. The recombinant Gag protein expressed in this study proved to be a very sensitive and specific reagent for screening for BIV infection. The BIV-specific nature of this reactivity is confirmed by the absence of reactivity seen in immune sera from animals infected with other bovine viruses, such as BVDV and BLV.

The Gag protein was chosen as the antigen for a variety of reasons. BIV Gag protein is highly immunogenic and contains sequences that are conserved among the lentiviruses. It has been demonstrated that Gag protein elicited an early, strong, and long-lasting immune response in cattle (25, 26). Antibodies to BIV Gag protein can be detected as early as 2 weeks after BIV infection and can last for at least 2 years. The recombinant Gag protein expressed in *E. coli* presents epitopes similar to those of the native viral protein and could be recognized specifically by sera from BIV-infected and -immunized animals. Based on sequence analysis of the BIV *gag* coding region, there are no obvious glycosylated sites (5). Therefore, it is unlikely that the mature Gag protein is glycosylated in vivo.

The *gag* gene from BIV has been cloned into a baculovirus expression system (17), but the protein expressed had a relatively low yield. Recently, the *gag* gene was expressed as a TrpE fusion protein in *E. coli* (3), and the protein yield was still relatively low. The bacterial part of the fusion protein in the TrpE system accounts for 50% of the total which might create problems for ELISA. The Gag protein expressed here was shown to be expressed at very high level, and the protein was purified to be near homogeneity as evidenced by a single band in Coomassie blue-stained gels (Fig. 2). Furthermore, the bacterial fusion part only accounts for 5% of the total protein, which greatly increased the specificity of the recombinant Gag protein used as an antigen in immunoassay.

Western blotting was the most widely used method to evaluate serological evidence of BIV in cattle serum samples (29). The Western blot assay was considered to be sensitive and reliable because its results were confirmed by PCR (29), and the Western blot method was chosen as the classic reference test in diagnosis of HIV infection (18). In this study, an ELISA method was developed for detection of BIV antibody in serum samples and its results were compared with those obtained by Western blotting. Ten known BIV-positive and -negative serum samples were tested for BIV antibodies using the two methods, which gave consistent results. However, there were some discrepancies for the field serum samples tested using the two methods (Table 1). There were eight ELISA-positive samples which were Western blot negative and three ELISA-negative samples which were Western blot positive. Those eight positive samples were considered weakly positive samples by ELISA because they had signal cutoff values of around 0.76 to 0.78, which was near the lower limit for positive samples (0.76). At the 90% confidence limit, those samples were considered to be negative. The three ELISA-negative samples had signal cutoff values of around 0.73, which were near the upper limit for the negative sample. At the 99% confidence limit, those samples were considered to be positive. All 11 samples were considered to be indeterminate by ELISA. These differences could be due in part to differences in test sensitivity. Generally, ELISA is more sensitive than Western blotting because it needs a relatively smaller amount of antigen in testing. The

TABLE 1. ELISA and immunoblotting results

| Sample | ELISA result | Immunoblot result | Sample | ELISA result | Immunoblot result | Sample | ELISA result | Immunoblot result |
|---|---|---|---|---|---|---|---|---|
| 1 | − | − | 48 | + | + | 95 | − | − |
| 2 | − | − | 49 | − | − | 96 | − | − |
| 3 | + | + | 50 | − | − | 97 | + | + |
| 4 | − | − | 51 | − | − | 98 | − | − |
| 5 | − | − | 52 | + | + | 99 | − | − |
| 6 | − | − | 53 | − | − | 100 | − | + |
| 7 | − | − | 54 | + | − | 101 | + | + |
| 8 | − | − | 55 | + | + | 102 | + | + |
| 9 | − | − | 56 | + | − | 103 | − | − |
| 10 | − | − | 57 | − | − | 104 | − | − |
| 11 | + | − | 58 | − | − | 105 | − | − |
| 12 | − | − | 59 | − | − | 106 | + | + |
| 13 | − | − | 60 | − | − | 107 | + | + |
| 14 | − | − | 61 | − | − | 108 | + | + |
| 15 | − | − | 62 | + | + | 109 | − | − |
| 16 | + | + | 63 | + | − | 110 | − | − |
| 17 | + | + | 64 | + | + | 111 | + | + |
| 18 | − | − | 65 | − | − | 112 | − | − |
| 19 | − | − | 66 | − | − | 113 | − | − |
| 20 | − | − | 67 | + | + | 114 | − | − |
| 21 | − | − | 68 | − | − | 115 | − | − |
| 22 | − | − | 69 | + | + | 116 | − | − |
| 23 | − | − | 70 | − | − | 117 | + | − |
| 24 | − | − | 71 | − | − | 118 | + | + |
| 25 | − | − | 72 | − | − | 119 | + | + |
| 26 | − | − | 73 | − | − | 120 | − | − |
| 27 | − | − | 74 | − | − | 121 | − | − |
| 28 | − | − | 75 | + | − | 122 | − | − |
| 29 | − | − | 76 | − | − | 123 | − | − |
| 30 | − | − | 77 | − | − | 124 | − | − |
| 31 | − | − | 78 | − | − | 125 | − | − |
| 32 | − | − | 79 | − | − | 126 | − | − |
| 33 | − | − | 80 | − | − | 127 | − | − |
| 34 | − | − | 81 | − | − | 128 | − | − |
| 35 | − | − | 82 | + | − | 129 | − | − |
| 36 | − | − | 83 | − | − | 130 | − | − |
| 37 | − | − | 84 | − | − | 131 | − | − |
| 38 | + | + | 85 | − | − | 132 | − | − |
| 39 | − | − | 86 | − | − | 133 | + | + |
| 40 | − | − | 87 | − | − | 134 | − | − |
| 41 | − | − | 88 | − | − | 135 | − | − |
| 42 | − | − | 89 | − | − | 136 | − | − |
| 43 | + | + | 90 | − | − | 137 | − | − |
| 44 | − | + | 91 | + | + | 138 | − | − |
| 45 | − | − | 92 | + | − | 139 | − | − |
| 46 | − | − | 93 | − | + | | | |
| 47 | − | − | 94 | − | − | | | |

[a] Samples 1 to 40 were from Kansas State University, and samples 41 to 139 were from Louisiana State University.

differences may be caused by the presence of other closely related retrovirus antibodies in field serum samples which may interfere with the BIV test. Since BIV is closely related to other retroviruses, antibodies against other retroviruses in serum samples might give false-positive results in Western blot and ELISA tests. A PCR test could overcome this problem by using unique primers designed for BIV. Since isolation of BIV from samples is very difficult, there is no way to confirm the true status of BIV infection. Thus, a combination of three assays, i.e., ELISA, Western blotting, and PCR, was necessary in order to confirm the indeterminate ELISA profiles. According to the recommendations of the World Health Organization, a combination of three tests is recommended for diagnostic testing for HIV (2). In HIV testing, a combination of three assays was reported to be necessary to avoid false-positive results (2).

In conclusion, the expressed protein has been used successfully as an antigen for ELISA. The ELISA established here is shown to be as specific as immunoblotting, and it has the advantage of being less time-consuming and thus more economical. Therefore, it provides a better means than the currently available serological tests to study BIV antibody status in cattle herds.

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