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Original article

Serological survey for bovine immunodeficiency virus in dairy cattle from Poland

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Abstract

A seroprevalence study of bovine immunodeficiency virus (BIV) was undertaken on 1,541 serum samples from Holstein cattle from 23 herds, located in different geographical regions of Poland. The analysis was performed using ELISA, with recombinant Gag protein of BIV as antigen. The average BIV prevalence was 4.9% in individual cattle, while the percentage of herds harboring at least one seropositive animal, was 82.6%. To demonstrate the correlation of BIV and bovine leukemia virus infection, all sera were analysed for BLV antibodies and there was only a slight association between both infections. Overall, these results show that BIV infection is present in dairy cattle in Poland at a prevalence rate found in other European countries.

Key words: bovine immunodeficiency virus, seroprevalence, ELISA

Introduction

Bovine Immunodeficiency Virus (BIV) is a lentiviral pathogen originally isolated from a cow showing persistent lymphocytosis, lymphoadenopathy, progressive weakness, and emaciation (Van der Maaten et al. 1972). A subsequent study has shown that BIV is genetically similar to human immunodeficiency virus (HIV-1) (Gonda et al. 1987) and this finding confirmed that it is important to evaluate the impact of BIV on the health of cattle. Since the first report of BIV in 1972, serological evidence for BIV infection has been reported in many countries worldwide with significantly higher prevalence in the USA (Gonda et al. 1994), moderate in Japan (Meas et al. 1998) and Australia (Burkala et al. 1999), and low in European countries and Canada (Polack et al. 1996, Jacobs et al. 1998).

Unlike other animal and human lentiviruses, the significance of BIV infection in cattle has not been clearly established, and it is still unknown whether BIV induces a specific syndrome or whether it renders animals more susceptible to other infections. It has been shown that BIV infection of cattle produces only moderate pathological effects (Carpenter et al. 1992). However, some reports have linked BIV to encephalitis, clinical immunodeficiency, and transient B-cell proliferation (Snider et al. 1997, Whetstone et al. 1997). Several reports confirmed that in herds where infection with bovine leukemia virus (BLV) was noted, cattle dually infected with BIV and BLV were found (Amborsky et al. 1989, StCyr Coats et al. 1994, Jacobs et al. 1995). The significance of this coinfection is not clear, and it was suggested that BLV might serve as cofactor for BIV replication (Snider et al. 1997). This assumption was additionally supported by

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the fact that BIV and BLV share common host cell tropisms, specifically for B-lymphocytes and monocytes (Heaton et al. 1998).

The present study reports a survey of dairy cattle from Poland for the presence of antibodies to BIV. In addition, the association between BIV and BLV infection was evaluated.

Materials and Methods

Serum sample collection

Blood samples were collected from 1,541 Holstein cattle, 2-10 years old, from 23 farms located in the Silesia, Wielkopolska, and Pommerania regions. The serum was obtained and stored at -20°C until use. A further 123 bovine sera, used to establish cut-off value and diagnostic sensitivity (Se) and specificity (Sp) of ELISA, were collected from cattle from herds in which BIV-infected animals were previously detected using TM peptide ELISA (Scobie et al.1999). The serological status of these sera was established by western blot analysis and TM peptide ELISA.

ELISA for detection of BIV specific antibodies

The 29 kDa recombinant Gag protein, used in this study as antigen for ELISA, was expressed from the pQE32-BIV plasmid vector, kindly supplied by Dr C.Wood, University of Nebraska, USA. ELISA was performed using the protocol described by Zheng et al. (2000). Briefly, ELISA plates (Immulon 2HB, Dynex Technologies) were coated with 400 ng of purified recombinant Gag protein, diluted in sodium carbonate buffer (pH 9.6), and incubated overnight at 4°C. The plates were washed with PBS containing 0.05% Tween 20 (PBST) buffer, blocked with 0.05% glycine in PBS, and incubated at 37°C for 30 min. The sera, diluted to 1:200 with 5% *E.coli* lysate in PBST buffer, were incubated at 4°C overnight and then at 37°C for 30 min in two replicates each. 100 μl of peroxidase-conjugated rabbit anti-bovine IgG (Sigma), diluted 1:5000 was added to each well and incubated at 37°C for 30 minutes. The plates were then washed and incubated at 37°C for 20 min with 100 μl of ABTS (Sigma) substrate for colour development. The absorbance was determined at 405 nm wavelength in an MR 5000 microplate reader (Dynatech). All sera were tested in duplicate and the mean value for each specimen was calculated as a sample-to-positive ratio [S/P-ratio = (OD sample-OD negative control)/(OD positive control-OD negative control)]. The ELISA was standardized to determine the desirable signal to noise ratio by using anti-BIV control serum obtained by i.v. inoculations of a 5-month old calf with 10^6 FBL cells, infected with clone R29-1203 of BIV, kindly provided by J. Miller, NADC, Aimes, USA. To assess the cut-off value and diagnostic Se and Sp of ELISA a receiver operating characteristic (ROC) analysis was performed using STATISTCA software, version8.0.

Western blot analysis of reference sera

Briefly, 22.5 μg of Gag protein was electrophoresed on 10% SDS-PAGE and transferred into a nitrocellulose membrane (Hybond-C, Amersham). The blots, after blocking with 2% bovine serum albumin (ICN) in TTBS buffer (20 mM Tris-base, 0.5 M NaCl, 0.1% Tween 20, pH 7.2), were then placed into a Mini-Protean II Mulitiscreen (BioRad), rinsed with TTBS, and incubated overnight at 4°C with sera diluted 1:100 in TTBS. The blots were then incubated with rabbit anti-bovine IgG (H+L) conjugated to horseradish peroxidase (Sigma) and bound anti-bovine IgG was detected by colour substrate 4-chloro-naphtol (ICN). Sera revealing the presence of the 29 kDa band were considered positive.

Serological Assays for BLV

ELISA Bovine Leukosis (Institut Pourquier, France) was used to detect antibodies to BLV in the sera of naturally infected cattle, according to the manufacturer's recommendations.

Statistical analysis

Seroprevalence of BIV in individual cattle and association between BIV and BLV infection were analysed using the test of differences between two proportions and the chi square test, respectively, using STATISTICA software, version 8.0.

Results

Optimization of ELISA

Preliminary titration experiments were conducted to determine optimum reagent concentrations and serum dilution using known BIV-positive and -negative sera. Out of 121 serum samples tested by western blot, BIV-specific antibodies were found in 23 samples while 98 were negative. The same serological

status of these samples was confirmed by TM-ELISA based on analysis performed at the Veterinary Laboratories Agency, Surrey, UK. These sera were then tested by developed ELISA and the results were used for ROC analysis. A single-graph ROC plot (Fig. 1) is a representation of test performance comparing true-positive results (Se) to false-positive results (1-Sp) over the entire range of results observed. ROC analysis demonstrated that an optimized cut-off of 0.312 by the ELISA would have a sensitivity of 100%. Under this condition, four negative sera were considered positive, thus diagnostic specificity was calculated as 94%. The plots were used also to assess the overall accuracy of the ELISA by calculating the area under the curve (AUC). The AUC= 0.983, and indicates that the ELISA was highly accurate.

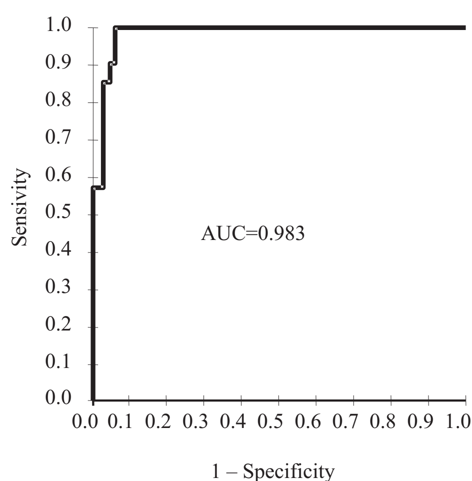

Fig. 1. Receiver-operating characteristic curve for the BIV ELISA constructed using entire ranges of results observed.

Analysis of BIV seroprevalence

Serum samples were analysed to detect antibodies against BIV and BLV, and the results are shown in Table 1. A herd was considered as positive when at least one cow was seropositive. Antibodies to BIV were detected in 19 (82.6%) out of 23 herds and the seroprevalence varied from 1.5% to 11.7% in particular herds. In four herds no BIV-positive animals were found.

Among 1541 cattle 77 (4.9%) were BIV positive and the number of seropositive animals versus seronegative was not statistically significant ($p<0.05$). When the same serum samples were tested for BLV specific antibodies were found in 353 (22.9%) samples. Twenty seven (1.8%) samples showed the presence of antibodies to both BIV and BLV.

To establish any association between BIV and BLV infection results of seroprevalence for both viruses were compared at herd and cattle level. As shown in Table 2, seropositivity to BLV was found in 16 herds, in which at least one infected cow was found. When compared to 19 herds in which BIV-infected cows were identified there was no significant association between the number of herds with incidence of BIV and BLV infection (χ^2 = 0.42, p = 0.517). There was a parallel increase in BIV and BLV-positive animals in some herds; however, there was only a slight association between BIV and BLV infection (χ^2 = 6.85, $p<0.01$).

Discussion

The large-scale seroprevalence study of BIV infection has been hampered by the lack of an effective and simple serological ELISA. Zheng et al. (2000) first reported the use of a recombinant BIV Gag protein-based ELISA that can be used for such an analysis. Our study has adopted and modified such an ELISA in order to investigate the prevalence of BIV infection in dairy cattle in Poland and the possible association between BIV and BLV infection. The establishment of a reliable cut-off value for a diagnostic test is essential to characterize its capability to differentiate populations of infected and uninfected individuals. Since there is no a gold standard to detect BIV infection (Orr et al. 2003), its estimation for the newly developed ELISA was done using ROC analysis. The estimated sensitivity was found to be 100%, since all 23 sera previously defined as positive by western immunoblot were positive. Since these sera were also positive by TM-ELISA derived from the FL112 isolate (Scobie et al. 1999), the conclusion can therefore be made that our ELISA is ideal for BIV serodiagnosis. In this study, the ELISA achieved 94% of specificity and this was slightly higher than obtained by Zheng et al. (2000). This enhanced specificity is probably due to the modification, in which all sera tested were preincubated with *E. coli* lysate. This allowed the removal of unspecific reactions against *E. coli* protein, the problem currently encountered in ruminant sera through serological analysis.

BIV seroprevalence was examined on sera collected from distinct geographical regions. The reasons underlying the sample collection in Silesia, Wielkopolska and Pommerania were multifold. The cattle industry in Poland is predominantly composed of dairy cattle with a special location of a number of herds in these areas. Although the mechanism of BIV transmission is unclear, the high density and close housing of cattle has been postulated as a means for a higher rate of infection in dairy herds (Snider et al. 1997). Since maintenance and breeding methods are

Table 1. Seroprevalences of antibodies against BIV and BLV in sera of cattle from 23 herds.

Farm no.	No. of cattle tested	No. of seropositive cattle (%)		No. of co-infected (BIV and BLV) (%)
		BIV	BLV	
1	40	1 (2.5)	0 (0.0)	0 (0.0)
2	60	2 (3.3)	0 (0.0)	0 (0.0)
3	74	0 (0.0)	0 (0.0)	0 (0.0)
4	70	4 (5.7)	2 (2.9)	0 (0.0)
5	93	7 (7.5)	11 (11.8)	2 (2.2)
6	60	4 (6.7)	1 (1.7)	1 (1.7)
7	44	4 (9.1)	26 (59.1)	4 (9.1)
8	25	1 (4.0)	4 (16.0)	0 (0.0)
9	20	0 (0.0)	2 (10.0)	0 (0.0)
10	25	0 (0.0)	7 (28.0)	0 (0.0)
11	55	5 (9.1)	20 (36.4)	2 (3.6)
12	45	2 (4.4)	39 (86.7)	1 (2.2)
13	42	0 (0.0)	37 (88.1)	0 (0.0)
14	120	14 (11.7)	43 (35.8)	6 (5.0)
15	45	2 (4.4)	45 (100)	2 (4.4)
16	45	2 (4.4)	38 (84.4)	2 (4.4)
17	25	6 (24.0)	6 (24.0)	2 (8.0)
18	105	3 (2.9)	0 (0.0)	0 (0.0)
19	106	9 (8.5)	0 (0.0)	0 (0.0)
20	148	3 (2.0)	0 (0.0)	0 (0.0)
21	201	3 (1.5)	0 (0.0)	0 (0.0)
22	49	3 (6.1)	46 (93.9)	3 (6.1)
23	44	2 (4.5)	26 (59.1)	2 (4.5)
Total	1541	77 (4.9)	353 (22.9)	27 (1.8)

Table 2. Results of serological survey for BIV and BLV among herds and individual cattle.

BIV status	BLV status					
	number of herds			number of cattle		
	positive	negative		positive	negative	
Positive	13	3	n=16	27	326	n=353
Negative	6	1	n=7	50	1138	n=1188
Total	19	7	n=23	77	1464	n=1541

different between dairy and beef cattle, a higher BIV seroprevalence in dairy cattle was noted (Amborsky et al. 1989, Polack et al. 1996). Antibodies to BIV were found in 4.9% of cattle, and this is comparable to the prevalence noted in other European countries: 4% in France (Polack et al. 1996), 6.6% in Germany (Muluneh 1994), 2.5% in Italy (Cavirani et al. 1998), and 5.5% in Great Britain (Scobie et al. 2001). However, the number of blood samples examined here represents a relatively small population of dairy cattle, and may introduce a bias in the estimates of BIV infection. Despite the fact that only 23 herds were tested, an inter-herd prevalence of 82.6% was similar to that found in Australia (Burkala et al. 1999), but was much higher than noted in France and Italy (Polack et al. 1996, Cavirani et al. 1998). Such relatively high prevalence indicates the risk of BIV spreading within herds and the possible future increase in infected individuals. The highest seroprevalence was noted among herds located in the Wielkopolska region as compared to others, and this can be related to the massive import of cattle in the early 90s from Germany and the Netherlands, i.e. countries where BIV infection was noted (Horzinek et al. 1991, Muluneh 1994).

In our study the occurrence of BIV seropositive cattle was not associated with seropositivity to BLV; however, dually infected cattle were found in 56% of herds and an increase in the number of BIV-infected cattle in parallel with BLV prevalence was noted. Thus, our results agree with previous reports (Jacobs et al. 1995, Hirai et al. 1996, Meas et al. 1998) on the lack of any epidemiological associations between the two infections. This may result in the lack of clinical syndromes in experimentally BIV/BLV infected cattle

(Straub et al. 1999) and the lack of lymphocytosis in experimentally and naturally co-infected cows (Cavirani et al. 1998, Isaacson et al. 1998). Nevertheless, dual infection with BIV/BLV was found in 2% of cattle, ranging from 1.7% to 9.1% in particular herds. Whether both viruses in co-infected individuals can act complementary to each other remains to be elucidated. Studies on humans infected with HTLV-I/HIV showed that HTLV-I may serve as cofactor during HIV infection, leading to an enhancement of virus replication, probably through cross transactivation (Moriuchi et al. 1998, Szabo et al. 1999). On the other hand, it has been shown that BIV infection influences the transmission of BLV from mothers to offspring and modulates BLV pathogenicity in experimentally infected sheep (Hirai et al. 1996, Meas et al. 2002).

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