J. Vet. Med. B 42, 385–390 (1995)
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ISSN 0931–1793

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Bovine Leukaemia-virus Infection in Costa Rica

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With 2 figures and 1 table

(Received for publication July 8, 1994)

Summary

The geographical distribution of bovine leukaemia-virus (BLV)-infected herds in Costa Rica and the isolation of BLV from cases of enzootic bovine leukosis are presented. It was found that BLV is prevalent throughout the country, affecting mostly dairy cattle (*Bos taurus*) located in the highlands around the central valley of Costa Rica. By contrast, beef cattle (*Bos indicus*) raised in the lowlands were affected to a lesser extent. One out of four isolates of BLV obtained from local cattle with lymphosarcomatous tumours was analysed and had similar physical, serological and biological characteristics to a reference strain of BLV isolated in the USA. However, different patterns of recognition of BLV-protein p24 were observed among naturally infected cattle using the western-blotting technique.

Introduction

Enzootic bovine leukosis (EBL) is the most frequent neoplasm of lymphatic tissue in cattle. The etiological agent is an exogenous retrovirus known as bovine leukaemia virus (BLV; BURNY et al., 1978). This agent can induce lymphocyte transformation and eventually, development of lymphosarcomatous tumours (FERRER, 1980). Immunologically relevant proteins of the BLV virion include the membrane glycoproteins gp51 and gp30 and the major core protein p24 (DESHAYES et al., 1977, 1980).

Several reports have shown that BLV-infection is widespread in cattle from the American continent (MARÍN et al., 1978; SAMAGH and KELLAR, 1982; DIGIACOMO, 1992). Economically, it is important due to restrictions in importation of seropositive animals and culling of clinically affected animals. Dairy herds are the most affected by BLV-infection and, in America, no indemnity programmes exist. Thus, all control programmes for EBL are herd based and strictly voluntary (MARÍN et al., 1978; RODRÍGUEZ et al., 1980; SHETTIGARA et al., 1986; CONTRERAS, 1991; DIGIACOMO, 1992). In Europe, state-funded control and eradication programmes for EBL have mainly been based on diagnosis by the agar-gel immunodiffusion test (AGIDT)

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followed by slaughter of infected animals (DIGIACOMO, 1992). Successful eradication of BLV-infection was attained in 1983 in the former Federal Republic of Germany where, after 5 years, there were fewer than 0.05 % BLV-positive cows (JOHNSON and KANEENE, 1992).

In Costa Rica, EBL was first diagnosed in 1976 at the School of Veterinary Medicine, Universidad Nacional (UNA), based on clinical and histopathological methods (GONZÁLEZ, 1977), and again in 1980 by AGIDT (RODRÍGUEZ et al., 1980). BLV has been reported to infect about 39 % of the animals of some dairy herds in the highlands of the central valley of Costa Rica (RODRÍGUEZ et al., 1980). This finding is important since the agricultural and livestock sectors are an important part of the Costa Rican economy, as they contribute to more than 20 % of the gross national product and generate almost 70 % of the total exports of the country.

This study was designed to determine the extent of BLV-infection in Costa Rica, both by serological detection and viral isolation from clinical cases. In addition, the recognition of BLV proteins by sera from naturally infected cattle was studied using the western-blotting technique.

Material and Methods

Cells, BLV-antigen and Sera

Chronically BLV-infected fetal-lamb kidney cells (FLK) and cloned bat-lung fibroblasts (BatCL2) were used as a source of viral antigen. Antigens were prepared from infected cell supernatants by ultracentrifugation (VAN DER MAATEN and MILLER, 1976). Isolation of BLV from infected animals was achieved by co-cultivation of blood lymphocytes with uninfected bat-lung fibroblasts. Virus infection was demonstrated by syncytia induction assay in human embryonic lung cells (HEL299), performed as described by GRAVES and FERRER (1976) and FERRER and DIGLIO (1976), respectively.

BLV-positive and BLV-negative control bovine sera, anti-bovine viral-diarrhoea-virus (BVDV) serum, and anti-bovine respiratory-syncytial-virus (BRSV) serum were obtained from the University of Pennsylvania, PA, USA. Local positive control BLV-sera were obtained from the EBL tumour-bearing cattle. Test sera were obtained from herds of beef cattle (zebu-crossbreeds) and from dairy herds (mainly Holstein and Jersey breeds) from areas of low and high BLV-prevalence, respectively (JIMÉNEZ, 1981; DUCREUX et al., 1987). Sera were also collected from 1983 to 1993 through the diagnostic service provided to the farms by the Virology Laboratory of the School of Veterinary Medicine, UNA. Most of these sera were from animals that required BLV testing for export purposes or from farmers and breeders who wanted to eradicate BLV from their herds.

Virus Characterization

Electron microscopy, SDS-PAGE, sucrose-gradient centrifugation, and CsCl buoyant-density centrifugation were performed as described previously (BONILLA, 1985; DOLZ, 1990).

Serological Methods

The agar-gel immunodiffusion test (AGIDT) was performed using Leukassay B^R (Pittman Moore Inc., Mundelein, IL, USA). Additionally, an AGIDT and western-blotting technique were carried out as described previously (BONILLA, 1985; DOLZ, 1990).

Results

Distribution of BLV Infection

The results of the serological survey carried out at the Virology Laboratory from 1983 to 1993 are shown in Table 1. Of 22463 sera tested, 4153 (18.4 %) reacted BLV-positive by AGIDT. A total of 953 (51.0 %) out of 1867 herds tested had BLV reactors.

The distribution of BLV-infected herds and detection of bovines with lympho-sarcoma tumours followed the distribution of dairy farms in Costa Rica. Most clinical cases and the highest serological prevalence were found in the highland regions (over 1300 m above sea level).

Table 1. Serological detection of BLV-antibodies with the AGIDT of bovines from different provinces of Costa Rica in the period 1983–1993

Province	Cattle Reacted/tested (%)	Herds Reacted/tested (%)
Alajuela	1975/9300 (21.2)	425/796 (53.3)
Cartago	871/5542 (15.7)	216/376 (57.4)
Guanacaste[1]	204/2035 (10.0)	49/167 (29.3)
Heredia	523/2040 (25.6)	114/207 (55.0)
Limon	128/625 (20.4)	25/38 (65.7)
Puntarenas[1]	66/1125 (5.8)	25/97(25.7)
San Jose	384/1796 (21.3)	99/186 (53.2)
Total	4153/22463 (18.4)	953/1867 (51.0)

[1]Predominantly *Bos indicus* cattle (*Bos indicus*/*Bos taurus* = 20/1)

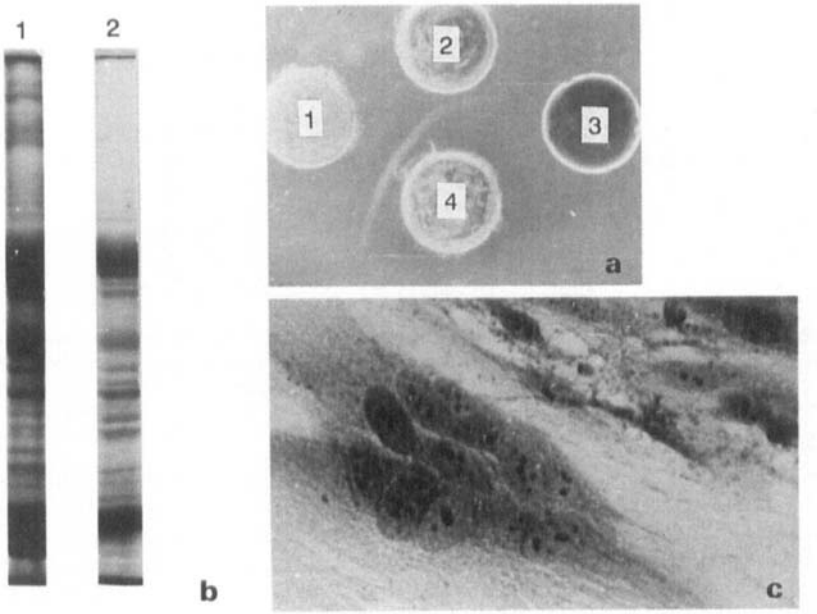

Fig. 1. Characterization of BLV isolated in Costa Rica (BLV-CR): a. Immunodiffusion analysis: 1. BLV-purified from chronically infected FLK cells; 2. BLV-CR isolated in batCL2 cells; 3. Culture fluids from non-infected batCL2 cells; 4. Serum from a BLV-infected cow; b. SDS-analysis of: 1. BLV purified from batCL2 culture fluids; 2. BLV-CR; c. Syncytia formation by BLV-CR in HEL299 cells

Isolation and Characterization of BLV

BLV was isolated from lymphocytes of four Costa Rican leukaemic and tumour-bearing cows. One of these isolates was further characterized. This isolate had a buoyant density of 1.18 g/cm^3 and gave a reaction of total identity by immunodiffusion test with BLV obtained from chronically infected FLK or BatCl2 cells (Fig. 1a). This virus isolate reacted by western blotting with positive BLV reference sera, as well as with sera from local tumour-bearing cattle, but not with sera against BVDV or BRSV

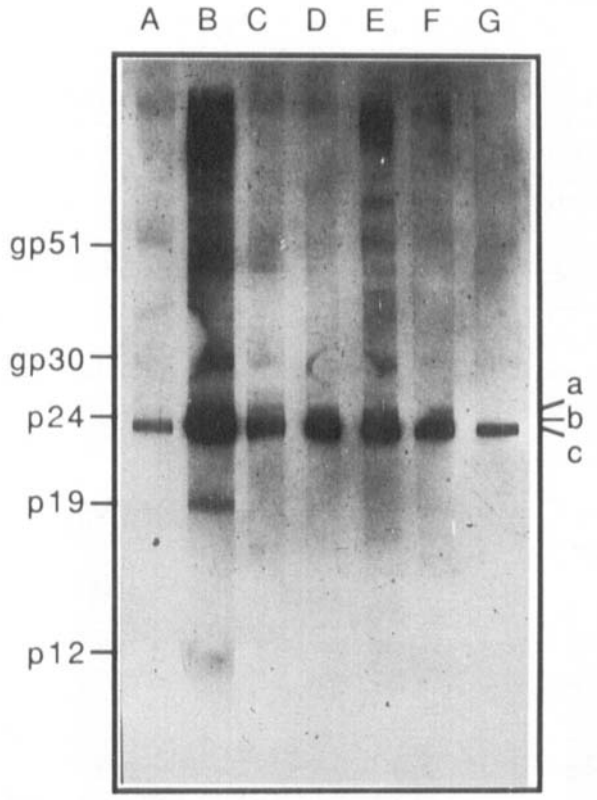

Fig. 2. Western-blotting patterns of BLV-proteins detected with sera from different tumour bearing cattle (A–G). The differential reactivity against the p24 region is shown (a–c). Immunodetection was carried out with I-125-labelled conjugate

(data not shown). The protein pattern in SDS-PAGE of the isolate was similar to that of BLV obtained from chronically infected FLK or BatCL2 cells (Fig. 1b). The virus isolate also induced syncytia typical of BLV in HEL299 cells (Fig. 1c).

Seven sera from immunodiffusion positive and tumour-bearing cattle recognized several BLV proteins in the western-blotting technique (Fig. 2). Although all sera tested reacted against the p24 protein, sera from certain animals resolved one, two, or three protein bands with molecular weights close to 24 K. The gp51 and gp30 antigens were also recognized by all sera tested; p15 and p12 antigens were recognized only by one serum. None of these protein bands reacted with bovine sera against BVDV and BRSV (data not shown).

Discussion

This study showed that BLV-infection is widely distributed throughout Costa Rica. The results show a high prevalence of BLV-infection in dairy herds, represented mainly by Holstein and Jersey breeds, distributed mostly on the highlands around the central valley, where more than 50 % of the dairy farms are located. In contrast, a very low prevalence of BLV-infection was observed in beef cattle (mostly of zebu-cross breeds) located in the lowlands of Costa Rica. Of all seven provinces of Costa Rica, Puntarenas and Guanacaste had the lowest prevalences. In terms of BLV-infected

herds, over 50 % of all tested herds had reactors. Again, only the provinces of Puntarenas and Guanacaste had a low percentage of infected herds. This could be explained by the fact that most of the cattle in these provinces are beef cattle, which are kept for a shorter duration than dairy cattle. The difference of BLV-prevalence in the highlands and lowlands of Costa Rica is probably not due to the genetic background of the bovines or to the geographical and climatic conditions, but to the fact that dairy cattle are kept for longer periods of time (more than 8 years in Costa Rica), thus allowing the development of the disease. Moreover, dairy cattle are kept in close contact and receive intensive husbandry, which increases the risk of transmission. In these herds, the management, iatrogenic factors and bloodsucking vectors would favour the transmission of BLV.

One BLV isolate from Costa Rica showed no antigenic difference with the reference strain originally isolated in the USA. In addition, no differences were observed in the electrophoretic patterns of BLV proteins and in the ability to induce syncytia between the costarrican and the US BLV isolate. Some small differences in the recognition pattern of BLV p24 protein were observed, when sera from naturally infected Costa Rican tumour-bearing cattle were tested in western blotting. These differences were observed both with the US and the Costa Rican isolates of BLV. These differences in the molecular weight could be associated with slight structural variations in the polypeptide chain (different epitopes), which are recognized differently by individual cattle. Although other post-translational modifications of p24 have not been reported, they cannot be excluded. It may be that the molecular heterogeneity observed is a result of tissue-culture conditions or virus degradation during purification (WALKER et al., 1987).

In conclusion, it has been established that BLV is highly prevalent in Costa Rica and that the virus isolate is similar to those in other countries where the disease is endemic. Most infected animals are from dairy herds. However, due to the rapid increase of double-purpose farms in the provinces of Guanacaste and Puntarenas, it is possible that BLV-infection will increase in these areas in future years, since there is no regulation of movement or official control of BLV-infected animals within Costa Rica. Control programs must, therefore, be established, particularly in herds with genetically valuable animals, and regulatory measures must be established to avoid further dissemination of BLV-infection among beef and double-purpose cattle.

Acknowledgements

The authors wish to thank Prof. Dr. Hartmut KRAUSS, Institut für Hygiene und Infektionskrankheiten der Tiere, Justus-Liebig Universität, Gießen, Germany, for his help and support, and the Clinical and Pathology Department of the Veterinary School, UNA, for its assistance. G. DOLZ and E. MORENO were research fellows from the Deutscher Akademischer Austauschdienst, (DAAD), Germany, during this investigation, which was supported by DAAD, Germany; International Foundation of Science (IFS), Sweden; International Atomic Energy Agency (FAO-IAEA), Austria; and the Vicerrectoría de Investigación from the Universidad de Costa Rica and the Universidad Nacional. The authors are also grateful to Dr. J. FERRER, University of Pennsylvania, Philadelphia, PA, USA, for supplying sera used in this study.

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