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Molecular detection of bovine leukosis virus in naturally infected dairy and dual-purpose cattle in Mexico

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Abstract

The objective of this study was to determine the prevalence of bovine leukosis virus (BLV) in specialized and dual-purpose dairy cows located in the central zone of Veracruz state in Mexico, using endpoint polymerase chain reaction (PCR). The study population consisted of 307 specialized dairy cows and 95 dual-purpose cows from 13 municipalities located in the study area. All cows were apparently healthy and ≥ 3 years old. Cows were stratified by age (3 - 5, 6 - 8 and ≥ 9 years). The overall prevalence of infection was 6.96%; the calculated prevalence in dairy cows was 7.82% and in dual-purpose cows it was 4.21%. The municipality with the highest proportion was Acajete (14.28%), followed by Huatusco and Tomatlán (11.53%). The association analysis confirms the infection's independence to the cows' productive purpose. The results by age strata were 3 - 5 (4.60%), 6 - 8 (8.00%) and ≥ 9 (18.40%) with $X^2 = 9.96$, with an odds ratio of 4.68 for the stratum ≥ 9 years with a significant difference. The present study determined the prevalence of proviral DNA of BLV in dairy and dual-purpose cows in six municipalities in the central zone of Veracruz state, Mexico, using endpoint PCR.

Introduction

Bovine leukosis as a disease harming cattle health is caused by a retrovirus belonging to the *Retroviridae* family, specifically an oncogenic member of the *Deltaretrovirus* genus. It is distributed worldwide and primarily affects dairy herds, where it has been associated with detrimental effects on production and reproduction.[1] The clinical manifestation of bovine leukosis is persistent lymphocytosis (PL) due to the polyclonal proliferation of infected B lymphocytes, occurring in about 30.00 - 70.00% of affected animals.[2] In less than 10.00% of cases, bovine leukosis virus (BLV) infection may progress to leukemia preceding lymphosarcoma after an incubation period of 2 - 8 years.[3] All infected animals develop antibodies to the virus and remain infected for life, serving as a potential source of infection for healthy animals.[4]

Asymptomatic cattle with BLV infection are classified into two disease phenotypes based on their blood's PL. The high proviral load phenotype (HPL) is characterized by more than 100,000 copies of provirus μg^{-1} DNA, associating with PL. The second phenotype is characterized by a low proviral load (LPL) in blood, with less than 100 copies of provirus μg^{-1} DNA, making molecular detection (endpoint polymerase chain reaction (PCR) and real-time PCR) more challenging.[5] Previous studies have established an association between these disease phenotypes and major bovine histocompatibility complex class II polymorphisms in the *BoLA DRB3* gene, indicating a genetic potential for resistance/susceptibility to BLV.[6]

The BLV is transmitted by transferring cells with proviral DNA via blood, secretions and excretions from infected to susceptible animals. Natural transmission occurs primarily through horizontal transmission, related to the poorly performed management practices and hematophagous insects. Vertical transmission has also been reported through intra-uterine transmission and milk ingestion, although passive immunity protects the offspring.[7,8]

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Due to the economic impact of BLV on productive and reproductive performances in dairy cattle,[1] this study aimed to determine the prevalence of BLV infection in dairy and dual-purpose cattle in Veracruz state, Mexico.

Materials and Methods

Study design and area. A cross-sectional observational study in dairy cattle herds in 13 municipalities in the central zone of Veracruz state, Mexico, was carried out.

Animal selection and sample size. Lactating cows, three years old or older, of specialized dairy breeds (Holstein Friesian, Brown Swiss and Jersey) and dual-purpose cows (beef and milk productions) were considered for the study. Sample size was 387, estimated using Win Episcope 2.0 online program (http://www.winepi.net/menu1.php) for cross-sectional studies, with a 95.00% confidence interval, a prevalence of 50.00% and an error rate of 5.00%. The research was approved with number of 03/23 by Bioethics and Animal Welfare Commission of the Faculty of Veterinary Medicine and Animal Science of the University of Veracruz, Veracruz, Mexico.

Sample collection. Blood samples were collected from each animal from the jugular or coccygeal veins using 4.00 mL vacuum tubes with EDTA (Vacutainer®; Becton, Dickinson and Company, Sumter, USA). The samples were transported refrigerated at 4.00 ˚C to the Biological Medical Research Institute Laboratory, University of Veracruz, Veracruz, Mexico.

Molecular analysis. Mononuclear cells were obtained from approximately 4.00 mL of blood being centrifuged at 700 *g* for 5 min to separate plasma from cell pellet; the latter was then resuspended in saline solution. The suspension was poured into another tube containing 3.00 mL of Lymphoprep™ (STEMCELL Technologies, Vancouver, Canada). It was then centrifuged at 500 *g* for 20 min. The mononuclear cell layer was separated from the centrifugate, deposited in microtubes with 1.00 mL of sterile water and stored at – 20.00 ˚C.[9]

DNA extraction. Mononuclear cells obtained from blood samples were processed with Chelex® 100 (Sigma-Aldrich, St. Louis, USA). A total of 300 µL of 10.00% Chelex® 100 resin was placed in a 1.50 mL microtube, along with 300 µL of mononuclear cell suspension in sterile water. Microtubes were placed in a thermomixer (Eppendorf AG, Hamburg, Germany) at 56.00 ˚C for 30 min; upon completion, each of the microtubes was vortexed (Daigger Scientific, Inc., Vernon Hills, USA) for 10 sec, placed back in the thermomixer at 95.00 ˚C for 10 min (Eppendorf AG) and then centrifuged at 10,000 *g* for 5 min. Finally, the DNA supernatant was separated, placed in a 1.00 mL microtube and stored at – 20.00 ˚C.[10]

Polymerase chain reaction. Molecular analysis was performed by endpoint PCR technique to amplify the env gene region, encoding the gp30 glycoprotein of the virus envelope. The following primer sequences were used: F: 5'ACGCTTGATCACAGCAATTA and R: 5' TCTGCGCTACACT CAGTC, resulting in a 674-bp product. The DNA was used at a concentration of 100 ng μL^{-1} and mixed in a solution for a total volume of 25.00 µL consisting of 12.50 µL PCR Master Mix (2x; Thermo Fisher Scientific Inc., Waltham, USA) containing 0.05 U μL^{-1} Taq DNA polymerase, reaction buffer, 4.00 mM $MgCl_2$, 0.40 mM of each dNTP (dATP, dCTP, dGTP, and dTTP), 1.00 µL of primer F, 1.00 µL of primer R, 5.00 µL of DNA (500 ng μL^{-1}) and 5.50 µL DNAase-free water. The PCR reaction was carried out in a thermal cycler model 2,720 (Applied Biosystems, Carlsbad, USA) with the following conditions: Initial denaturation at 95.00 ˚C for 5 min, followed by 40 cycles consisting of denaturation at 95.00 ˚C for 40 sec, annealing at 54.00 ˚C for 40 sec and extension at 72.00 ˚C for 60 sec extended to 72.00 ˚C for 15 min for the final cycle.[11]

Statistical analysis. The data were processed by VassarStats program (http://vassarstats.net/) to determine proportions and 95.00% confidence intervals. The risk variables including breed and age were evaluated by odds ratio through Win Episcope 2.0 online program.

Results

The overall prevalence of BLV-positive cows was 6.97% (28/402). The location with the highest number of BLV-positive cows was La Joya, in the municipality of Acajete (13/91), where only dairy breeds were tested. This was followed by the municipalities of Huatusco (9/78), where samples were obtained from 46 dairy cows (7/9) and 32 dual-purpose cows (2/9), and Tomatlán (3/26), with 23 samples originating from dairy cows (3/3) and only three samples originating from dual-purpose cows. In Coscomatepec (1/8), only one dairy cow and seven dual-purpose cows (1/1) were sampled. Only dual-purpose cows were sampled in Puente Nacional (1/10), and finally, in Rafael Lucio (1/8), all sampled animals were dairy cows. No BLV-positive cases were identified in the municipalities of Las Vigas, Miahuatlán, Naolinco, Tlacolulan, Puente Nacional, Veracruz and Acatlán (Table 1).

The study also investigated the association between BLV positivity and the age of sampled animals. Chi-square analysis showed that the presence of BLV infection depended on the age of cows (p = 0.007). The highest proportion of positive cows (18.42%) was found in the stratum of nine years or older cows (Table 2). The calculated odds ratio indicated that the possibility of BLV infection was 4.68 times higher in cows over nine years old compared to the cows younger than nine years old, and this association was statistically significant ($p < 0.05$).

Breed risk variables for acquiring BLV infection were analyzed. Still, chi-square analysis did not indicate the presence of BLV infection to be dependent on productive purpose of cows (specialized dairy versus dual-purpose).

Table 1. Cows positivity for bovine leukosis infection being identified by PCR in the central zone of Veracruz state in Mexico.

Municipality	Samples	Positive samples (%)	95.00% confidence interval
Acajete (La Joya)	91	13 (14.28)	(0.08 - 0.23)
Huatusco	78	9 (11.53)	(0.05 - 0.21)
Tomatlan	26	3 (11.53)	(0.03 - 0.31)
Coscomatepec	8	1 (12.50)	(0.00 - 0.53)
Paso de Ovejas	10	1 (10.00)	(0.00 - 0.45)
Rafael Lucio	8	1 (12.50)	(0.00 - 0.53)
Total	402	28 (6.96)	(0.04 - 0.10)

Table 2. Distribution by age strata of cases positive for BLV infection being diagnosed by endpoint PCR.

Age (years)	Samples	Positive cows (%)	Odds ratio	95.00 % confidence interval
3 - 5	239	11 (4.60)	1.00	-
6 - 8	125	10 (8.00)	1.80	0.74 - 4.36
≥ 9	38	7 (18.42)	4.68	1.69 - 12.97
Total	402	28 (6.96)	-	-

$X^2 = 9.96$, df = 2.00, and $p = 0.007$.

Discussion

The prevalence of BLV infection in Mexico has been studied; but, there is limited information on distribution of BLV infection in the country. One study from 1987 has reported a seroprevalence of 15.78% in cross-breed beef cattle in the state of Veracruz using immunodiffusion in agar gel test, and an average seroprevalence of 9.83% in five states (Oaxaca, Tamaulipas, Veracruz, Puebla and Yucatan) in milk and beef-producing animals.[12] However, the animals sampled in this study came from a single herd and may not represent the overall prevalence in the state. In the present study using the endpoint PCR technique, the proportion of BLV-positive cows was 6.97% (28/402). Regardless of the difference in prevalence, the molecular analysis confirms current presence of BLV in Veracruz state's cows.

More recent studies have reported a higher prevalence of BLV infection using different diagnostic methods. For example, studies using enzyme-linked immunosorbent assay have estimated a prevalence of 66.00% in a production unit in Tizayuca Hidalgo in Holstein cows.[13] Molecular detection of BLV using PCR analysis has also been performed in different states of Mexico, with prevalences ranging from 4.40 to 58.00% in Holstein cows of various ages.[11,14]

Studies have reported varying prevalence rates of BLV infection in other countries. For example, prevalence of 27.90% in Chile, 30.70% in Bolivia, 33.30% in Venezuela, 42.30% in Peru, 54.70% in Paraguay, 60.80% in Brazil and 77.40% in Argentina have been reported using PCR.[5] In Colombia, a prevalence of 22.60% has been reported in animals with different productive purposes.[15]

This study included lactating specialized and dual-purpose dairy cows from recognized municipalities with substantial dairy production in Veracruz state. The highest percentage of positive samples was found in the municipality of Acajete (14.28%), and Holstein-Friesian was the predominant breed in the sampled herds. This breed has been genetically flagged as susceptible to BLV due to its association with HPL bovine leukosis infection phenotype associated with major bovine histocompatibility complex class II polymorphism of the *BoLA-DRB3* gene (*DRB3.2*22* allele),[6] (*DRB3*1501* allele).[16] In contrast, the positivity found in dual-purpose cows sampled in Huatusco municipality was lower; while, in Veracruz and Puente Nacional municipalities, it was null for crossbreed cows. The findings of this study further support the notion that cross-breeding with zebu breeds, possessing the *BoLA-DRB* gene polymorphism allele *DRB3.2*,[17] *DRB3*0902*,[18] is associated with protection against BLV infection and LPL phenotype.

The study also found that the proportion of BLV-positive animals increased with age,[8] being consistent with previous studies showing that older animals are more susceptible to the disease.[2,19,20] Continuous contact of cows with infected animals over time increases the probability of infection.[2] Management practices in dairy and dual-purpose herds can also be risk factors associated with the transmission of BLV.[7,8]

Overall, the study confirms the presence of BLV in cows in the state of Veracruz, Mexico, and highlights the need for further researches to understand better the distribution and prevalence of BLV infection in the country as well as the risk factors associated with transmission and strategies for control and prevention of BLV in dairy and beef cattle populations.

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Conflict of interest

The authors have no conflict of interests to declare.

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