Seroprevalence and risk factors associated with bovine leukemia virus (BLV) seropositivity in cattle herds from Ecuador

Seroprevalencia y factores de riesgo asociados a la seropositividad al virus de la leucemia bovina (BLV) en explotaciones de producción bovina en Ecuador

Seroprevalência e fatores de risco associados à soropositividade do vírus da leucemia bovina (BLV) em rebanhos bovinos do Equador

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Abstract

Background: Bovine leukemia virus (BLV) is the causative agent of enzootic bovine leukosis (EBL). This disease mainly affects cattle, causing severe economic losses to producers. **Objective:** To establish individual and herd seroprevalence and determine the risk factors associated with BLV seropositivity for dairy and dual-purpose cattle herds in Ecuador. **Methods:** A total of 2,668 serum samples from 386 herds were collected. A questionnaire, including variables related to cattle health, management and the environment was completed by each herd. A commercial blocking enzyme-linked immunosorbent assay (ELISA) test was used to determine seropositivity. A generalized estimating equation model (GEE) was developed to determine the factors associated with BLV seropositivity. **Results:** Individual seroprevalence of BLV infection in Ecuador was 17.3% ($CI_{95\%}$ = 15.86-18.74%). Herd prevalence was 37.8% ($CI_{95\%}$ = 33.0-42.6%), and intra-herd prevalence ranged between 12.5 and 100% (median: 37.5%). The risk factors associated with BLV seropositivity were artificial insemination (OR: 2,215; $CI_{95\%}$ = 1.402-3.501), concrete floors (OR: 2.178; $CI_{95\%}$ = 1.217-3.889), presence of wild ruminants (OR: 2.998; $CI_{95\%}$ = 1.788-5.027),

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and sampling season (wet; OR: 1.996; $CI_{95\%}$ = 1.140-3.497). **Conclusions:** Results indicate that BLV is widespread in cattle herds in Ecuador. In addition, the study suggests that a control program to fight BLV infection should focus on controlling the risk factors identified.

Keywords: *BLV; bovine leukemia virus; cattle; cows; EBL; enzootic bovine leukosis; epidemiology; prevalence; risk factors; ruminant; seroprevalence; seropositivity; virus.*

Resumen

Antecedentes: El virus de la leucosis bovina (BLV) es el principal agente etiológico causante de la leucosis enzoótica bovina (EBL). Esta enfermedad afecta a los bovinos causando grandes pérdidas económicas a los productores. **Objetivo:** Establecer la seroprevalencia y dispersión del BLV, así como los factores de riesgo asociados a la seropositividad en explotaciones lecheras y de doble propósito en Ecuador. **Métodos:** Se recolectó un total de 2.668 muestras de suero de 386 explotaciones. Se aplicó un cuestionario que incluyó variables relacionadas con la salud del hato, medidas de manejo, y características ambientales de cada explotación. Para los análisis serológicos se utilizó un test inmunológico ligado a enzimas (ELISA). Para definir los factores de riesgo asociados a la seropositividad a BLV se desarrolló un modelo utilizando ecuaciones de estimación generalizadas (GEE). **Resultados:** La seroprevalencia de BLV en Ecuador fue de 17,3% ($IC_{95\%}$ = 15,86-18,74%). La dispersión fue de 37,8% ($IC_{95\%}$ = 33,0-42,6%), y la prevalencia intra-hato alcanzó rangos entre 12,5-100% (media: 37,5%). Los factores de riesgo asociados a la seropositividad a BLV fueron: inseminación artificial (OR: 2,215; $IC_{95\%}$ = 1,402-3,501), piso de concreto (OR: 2,178; $IC_{95\%}$ = 1,217-3,889), presencia de rumiantes salvajes (OR: 2,998; $IC_{95\%}$ = 1,788-5,027), y temporada de muestreo (húmeda; OR: 1,996; $IC_{95\%}$ = 1,140-3,497). **Conclusiones:** Los resultados indican que el BLV se encuentra disperso en las explotaciones de Ecuador. Adicionalmente, se sugiere la implementación de un programa de control para la lucha contra el BLV, debiéndose considerar medidas que se enfoquen al control de los factores de riesgo identificados en esta investigación.

Palabras clave: *BLV; bovinos; EBL; epidemiología; factores de riesgo; ganado; leucosis enzoótica bovina; prevalencia; rumiante; seroprevalencia; seropositividad; virus; virus de la leucemia bovina.*

Resumo

Antecedentes: O vírus da leucemia bovina (BLV) é o principal agente causador da leucose enzoótica bovina (EBL). Esta doença afeta o gado causando graves prejuízos econômicos aos produtores. **Objetivo:** Estabelecer a soroprevalência e dispersão do BLV, assim como os fatores de risco associados à soropositividade nas produções leiteiras e de duplo propósito no Equador. **Métodos:** Um total de 2.668 amostras de soro de 386 explorações foram coletadas. Foi aplicado um questionário que incluía variáveis relacionadas à saúde do rebanho, medidas de manejo e ambiente para cada exploração. Para a análise sorológica foi utilizado um teste imunológico sobre enzimas (ELISA) para determinação da soropositividade. Para definir os fatores de risco associados à soropositividade a BLV, foi utilizado um modelo de equações estimativas generalizadas (GEE). **Resultados:** A soroprevalência de BLVno Equador é de 17,3% ($IC_{95\%}$ = 15,86-18,74%). La dispersão de 37,8% ($IC_{95\%}$ = 33,0-42,6%), e a prevalência intra-rebanho alcançou entre 12,5-100% (media: 37,5%). Os fatores de risco associados à soropositividade a BLV foram inseminação artificial (OR: 2,215; $IC_{95\%}$ = 1,402-3,501), chão de concreto (OR: 2,178; $IC_{95\%}$ = 1,217-3,889), presença de ruminantes selvagens (OR: 2,998; $IC_{95\%}$ = 1,788-5,027) e época da amostragem (úmida; OR: 1,996; $IC_{95\%}$ = 1,140-3,497). **Conclusões:** Os resultados indicam que o BLV se encontra disseminado nas explorações no Equador. Adicionalmente, o estudo pode contribuir para a implementação de um programa de controle para a luta contra o BLV, devendo-se considerar ações de controle dos fatores de risco identificados nesta investigação.

Palavras-chave: *BLV; bovino; EBL; epidemiologia; Equador; fatores de risco; gado; leucose enzoótica bovina; prevalência; ruminante; soroprevalência; soropositividade; vírus da leucemia bovina; vírus.*

Introduction

Bovine leukemia virus (BLV) is an oncogenic and lymphocytotropic Deltaretrovirus belonging to family *Retroviridae*, subfamily *Orthoretrovirinae* and genus *Deltaretrovirus*. It is the aetiological agent of enzootic bovine leukosis (EBL). This agent is phylogenetically related to human T-cell leukemia virus type 1 (HTLV-1; Gillet *et al.,* 2007; Rodriguez *et al.,* 2011). The EBL has been eradicated in many countries, mainly in the European Union (Nuotio *et al.,* 2003; Acaite *et al.,* 2007), but this disease is still routinely diagnosed in all Latin-American countries. Economic losses caused by EBL are mainly due to premature culling, reduction in milk production, and commercial restrictions with countries where official control has been implemented (Chi *et al.,* 2002). The BLV induces chronic infection, usually with persistent lymphocytosis, as well as death due to lymphosarcoma in 5-10% of the infected animals. Mortality is high in cattle older than 4-5 years. BLV is mainly transmitted horizontally through indirect exposure to biological fluids containing previously infected B-lymphocytes. Colostral antibodies show high inhibitory activity until day 3 of lactation (Rodriguez *et al.,* 2011; Konishi, *et al.,* 2018).

The presence of horseflies, poorly sanitized dehorning procedures, colostrum feeding, artificial insemination, embryo transfer, blood transfer, and improper practices can lead to iatrogenic transmission and have been described as risk factors associated with BLV infection (Hopkins and DiGiacomo, 1997; Kobayashi *et al.,* 2010). Therefore, eradication and control of BLV are based on early diagnosis culling, segregation, and elimination of carriers and improvement of farm management and biosecurity (Nuotio *et al.,* 2003; Rodriguez et al., 2011; Ruggiero *et al.,* 2019).

There are no EBL eradication programs in Ecuador and control measures are not widely applied. Therefore, this study aimed to establish individual and herd prevalence of BLV, which would increase visibility of the infection in this geographic area, and determine the risk factors associated with BLV seropositivity in dairy and dual-purpose cattle herds from Ecuador.

Materials and Methods

Study design

A cross-sectional study was carried out to determine BLV seroprevalence in Ecuador. Extensive production systems are mainly used for cattle farming in Ecuador (most farms do not have basic infrastructures; such as stables). Autochthonous/creole racial groups (e.g. Chusco, galapagueño, jaspeado manabita) or crossbred (e.g. Creole-Holstein, Jersey-Holstein) are the most abundant cattle breeds in this country. Few farms have pure breeds such as Jersey, Holstein, Ayrshire, Charolais, Brown Swiss, among others (FAO, 2020).

In these geographical and productive conditions, the study was carried out in dairy and dual-purpose (dairy-beef) farms located in the main milk-producing provinces of Ecuador (i.e., Azuay, Chimborazo, Cotopaxi, Manabí, Santo Domingo, Pichincha, Tungurahua, and Zamora Chinchipe).

Cattle older than 6 months constituted the study population, while all cattle from dual-purpose and dairy herds from Ecuador were the target population. A herd was considered as dairy or dual-purpose when milk was collected and sold.

A herd was considered positive to BLV, and therefore, infected if at least one animal was seropositive. An epidemiological software (Win Episcope 2.0) was used to calculate the sample size. A minimum sample size of 386 herds was obtained using 50% of expected prevalence (there are no previous studies in the area), a 95% confidence level, and an acceptable error of 5%.

Due to the lack of detailed cattle herd identification information in Ecuador, the following procedure for herd selection was used: First, stratified sampling was performed, according to the number of herds in each

province regarding the population in all of the provinces included in the study. Second, each province was divided into blocks of twenty-five square kilometers. Third, the blocks were randomly selected. Following the order provided by the software, all of the farms in the first block were visited (cluster sampling), and then all of the farms in the second block, and so on until the total number of herds per province was obtained.

A total of 386 herds were sampled. Sample size for detecting if a herd was infected was also calculated with epidemiological software using an intra-herd prevalence of 35% (based on preliminary studies), a herd size of 10,000 (greater than the largest herd in Ecuador), and a significance level of 95%, yielding a minimum sample size of 8 animals per herd. As most herds are smaller in the study area, (>80% of the herds with <100 animals), sample size showed better sensitivity to detect infected herds (with the same number of samples, sensitivity is greater if herd size is smaller). Among farms with herd size of eight animals or less, all animals older than six months were sampled. This design has been used in similar studies in Ecuador (Saa *et al.,* 2012).

Serological analysis

Blood samples (10 ml) from each animal were collected by puncture in the caudal vein with a needle using tubes without anticoagulant (Vacutainer®, Becton-Dickinson). Serum samples were stored at -25 ˚C until analyses.

Presence of antibodies to BLV was tested using a commercial blocking enzyme-linked immunosorbent assay (ELISA Ingezim BLV Compac2.0, Madrid, Spain) based on two monoclonal antibodies against viral gp51. This test allows detecting antibodies against BLV in bovine serum or milk (individual or pooled). Plates were coated with BLV gp51 and bound to the plate trough two specific monoclonal antibodies against gp51 virus protein. After adding the sample to the well, if it contains specific antibodies against the virus, they will bind to the antigen absorbed on plate. In contrast, if the sample does not contain specific antibodies,

they will not bind to the antigen. Adding the substrate in the presence of peroxidase develops a colorimetric reaction. According to the manufacturer, diagnostic test sensitivity and specificity were 100%, so true prevalence was considered equivalent to the apparent prevalence.

Data collection

Considering variables potentially associated with BLV infection, a structured questionnaire was completed in each herd by direct interview with the farmer. Our observations were supported with feedback from the farmers. The variables included were grouped by topics: Cattle-related data, general data about the farm, management measures, reproduction system, feeding, facilities, introduction of the infection, and animal health.

Statistical analysis

An initial selection of the explanatory variables was performed using the Chi-square test to determine the risk factors associated with infection, selecting variables associated with BLV seropositivity with $p<0.05$. Next, a Phi test was used for dichotomic variables to determine collinearity between selected explanatory variables. If Phi was $> (0.4)$, only the variable more logically associated with BLV seropositivity was retained (the *p*-values associated with Phi were <0.001 in all cases). On the other hand, an association between BLV seropositivity and quantitative variables was determined using a Kolmogorov-Smirnov test for independent variables (variables were selected when $p<0.05$). In a second step, correlations between independent quantitative variables were evaluated by an r of Pearson. If r was $> (0.4)$, only the variable more logically associated with BLV seropositivity was retained.

The effect of the previously selected exploratory variables on the dependent variable was investigated using a generalized estimating equation (GEE) model. The BLV seropositivity was the dependent variable, and the herd was included as a random effect. Changes in the OR greater than 20% were considered indicative of

confounding (associations could be due to a third variable associated with both the dependent and the independent variable). The model was re-run until all of the remaining variables presented with statistically significant values (the likelihood-ratio Wald's test, $p<0.05$), and a potential causal relationship with the dependent variable existed. The choice of the best model was based on quasi-likelihood under the independence model criterion (QIC). The statistical analysis was performed using software (SPSS v15.0). The maps were developed with a commercial software (ArcMap^{TM} 9.3).

Map design

To obtain the probability map of BLV seropositivity occurrence in Ecuador, a model of maximum entropy ecological niche model (MaxEnt; version 3.4.1; Phillips, 2006) was used. Similarly, to determine the regions of maximum probability of prevalence, 139 notifications of seropositivity were used, distributed in two subgroups.

Nineteen independent variables with a resolution of 1 km sourced from WorldClim were used (Hijmans *et al.,* 2005). The model was calibrated with a default convergence threshold, regularization of 1, and a number of interactions of 5000. A logistic model was used to obtain values between 0 and 1. Those variables with high weight from the 19 climatic variables were identified, and Spearman's coefficient was calculated to determine the correlation between variables. If the Spearman's Rho was >0.6, one of the variables was eliminated. Finally, four variables were selected (Bio1: annual mean temperature; Bio 4: seasonality of the temperature-standard deviation; Bio12: annual rainfall; and, Bio 13: rainfall in the wettest month).

The final model was evaluated using cross-validation with 75% of the samples for training (105 notifications) and 25% for validation (34 notifications). In this way, the yields were evaluated using the area under the curve (AUC). The final model was used to generate the BLV seroprevalence map, using QGIS 2.14.

Results

A total of 2,668 serum samples from 386 herds were taken. Individual seroprevalence of BLV was 17.3% (461/2,668; $CI_{95\%}$ = 15.86-18.74%), while herd prevalence was 37.8% (146/386; $CI_{95\%}$ = 33.0-42.6%). Intra-herd prevalence ranged from 12.5 to 100% (median: 37.5%).

In the univariable analysis (Chi-squared analysis), seroprevalence was significantly higher in dairy herds (20.4%) compared to dual-purpose herds (11.1%). Significantly higher seropositivity was also found in crossbred cattle (23.1%) as compared to pure breed and creole cattle jointly (14.1%; Table 1).

Table 1. Nominal variables associated (p<0.15) with bovine leukemia virus (BLV) seropositivity at herd level among dairy and dual-purpose cattle herds in Ecuador.

Variable	Category	Total (% BLV seropositivity)	Chi-squared *p*
Herd type	Dairy farms	362 (20.4)	<0.001
	Dual-purpose	99 (11.1)	
Creole breed	Yes	170 (10.6)	<0.001
	No	291 (27.4)	
Crossbreed	Yes	216 (23.1)	<0.001
	No	245 (14.1)	
Cleanliness of facilities	Good	237 (19.9)	<0.001
	Bad	224 (15.2)	
Division of pastures	Yes	317 (22.2)	<0.001
	No	144 (11.6)	
Natural mating	Yes	231 (11.8)	<0.001
	No	230 (32.4)	

Artificial insemination	Yes	225 (33.4)	<0.001
	No	236 (11.8)	
Synchronization of births	Yes	136 (34.4)	<0.001
	No	325 (14.3)	
Colostrum used to feed calves	Yes	340 (18.8)	0.003
	No	121 (14.1)	
<u>Ad</u> <u>libitum</u> *feeding*	Yes	116 (11.6)	<0.001
	No	345 (20.7)	
Total absence of facilities	Yes	134 (15.0)	0.029
	No	327 (18.4)	
Livestock chute	Yes	222 (23.1)	<0.001
	No	239 (14.0)	
Milking parlor	Yes	206 (37.9)	<0.001
	No	255 (12.0)	
Stables	Yes	209 (25.2)	<0.001
	No	252 (13.7)	
Ground-like bed	Yes	103 (9.4)	<0.001
	No	358 (22.7)	
Wood chips bed	Yes	8 (50.0)	<0.001
	No	453 (17.1)	
Concrete floor	Yes	117 (31.8)	<0.001
	No	344 (15.0)	
Footbaths	Yes	16 (50.0)	<0.001
	No	445 (16.9)	
Presence of sheep	Yes	89 (14.1)	0.017
	No	372 (18.3)	
Presence of wild ruminants	Yes	130 (29.6)	<0.001
	No	331 (14.8)	
Adjacent cattle farms	Yes	222 (19.4)	0.011
	No	239 (15.7)	
Purchase of animals	Yes	67 (26.9)	<0.001
	No	394 (16.3)	
Replacement of animals	Yes	319 (21.6)	<0.001
	No	142 (11.9)	
Purchase and replacement of animals	Yes	157 (13.9)	<0.001
	No	304 (19.8)	
Disinfection of the umbilical cord	Yes	164 (19.7)	0.025
	No	297 (16.2)	
Deworming of calves	Yes	320 (19.4)	<0.001
	No	141 (13.9)	
Deworming of adults	Yes	313 (18.9)	0.005
	No	148 (14.6)	
Quarantine	Yes	121 (22.3)	<0.001
	No	340 (16.0)	
Andean region	Yes	316 (21.7)	<0.001
	No	145 (11.9)	
Sampling season	Wet	87 (15.5)	<0.001
	Dry	374 (34.7)	

BLV seroprevalence significantly increased in herds with more than 40 animals (20.6 vs. 13.3% in herds <40 animals). The distribution of BLV seropositivity among provinces is shown in Table 2.

Table 2. Distribution of bovine leukemia virus (BLV) herd seroprevalence by province in Ecuador.

Province	Bovine leukemia virus status		Total
	Positive* (%)	Negative (%)	
Azuay	31 (53.4)	27 (46.6)	58
Chimborazo	14 (31.8)	30 (68.2)	44
Cotopaxi	22 (57.9)	16 (42.1)	38
Manabí	25 (23.6)	81 (76.4)	106
Pichincha	26 (49.1)	27 (50.9)	53
Santo Domingo	8 (22.9)	27 (77.1)	35
Tungurahua	7 (22.6)	24 (77.4)	31
Zamora Chinchipe	13 (61.9)	8 (38.1)	21
Total	146 (37.8)	240 (62.2)	386

*Herds with at least one seropositive animal.

The highest seroprevalence (>50%) was found in Azuay, Cotopaxi, and Zamora Chinchipe provinces. Figures 1 and 2 show prevalence and distribution of BLV seropositivity in Ecuador at individual and herd level, respectively. Distribution of BLV seropositivity was widespread. Infection was present in all provinces, ranging from 22.6% in Tungurahua to 61.9% in Zamora Chinchipe.

Results from the univariable analysis are shown in Tables 1 and 3. No confounding factors or potentially relevant interactions between independent variables were observed in the final model. The GEE model included sampling season (wet), artificial insemination, concrete floor, and presence of wild ruminants as risk factors associated with BLV seropositivity (Table 4).

Rainfall in the wettest month (46.9%), temperature (23.4%), annual precipitation (15.3), and temperature seasonality (14.4%) were identified as climatic factors with the greatest predictive value for occurrence of BLV seropositivity in Ecuador. The AUC of the data for training was 0.97, while that for validation was (0.888). Thus, the model agreed exceptionally well for both training and validation data.

The results show that probability of seropositivity increases for annual rainfall between 750 to 900 mm, with high monthly variations (rainfall in the wettest month between 75 to 120 mm). Mean annual temperature was between 10 to 15 degrees, ranging between 6.2 and 7.5 degrees (standard deviation; Figures 3 and 4).

Table 3. Quantitative variables selected in the univariable analysis for bovine leukemia virus (BLV) seropositivity in dairy and dual-purpose cattle farms from Ecuador (p<0.05).
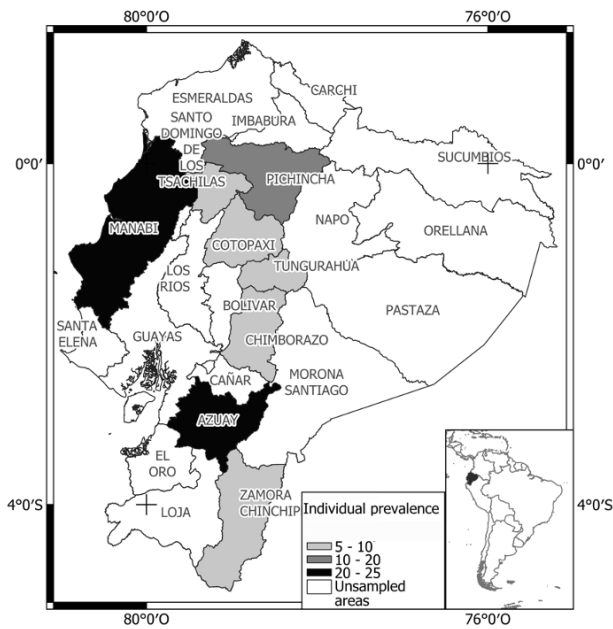
Variable	Mean	Median	Mode	Standard error	Min.[1]	Max.[2]	K-S Z[3]	p
Animal age (months)	46.2	48	48	31.3	3	180	3.60	<0.001
Number of cattle	82.8	40	60	146.7	2	1600	3.29	<0.001
Number of cows	24.1	13	5	30.8	0	230	3.67	<0.001
Number of calves	19.0	9	3	38.0	0	500	2.15	<0.001
Farm size (ha)	110.6	30	40	305.8	<1	3200	3.34	<0.001
Hectares used by cattle	82.4	25	30	235.0	<1	3200	3.04	<0.001
Terrain slope	16.1	15	0	15.2	0	60	1.42	0.036
Altitude (m)	1639.7	1767.07	200	1348.4	2.5	3914.8	5.67	<0.001
Calf age at weaning (months)	4.0	5	<1	3.4	<1	88	4.59	<0.001
Annual mortality rate of adults	4.4	1	<1	7.7	<1	45	3.94	<0.001

[1]Minimum value. [2]Maximum value. [3]Kolmogorov - Smirnov Z.

Figure 1. Animal-level seroprevalence of bovine leukemia virus (BLV) in several Ecuador provinces.
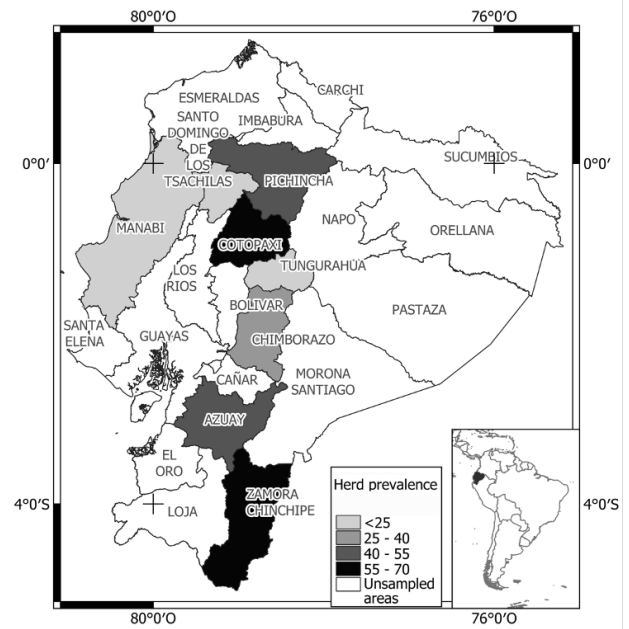
Figure 2. Herd-level seroprevalence of bovine leukemia virus (BLV) in several Ecuador provinces.
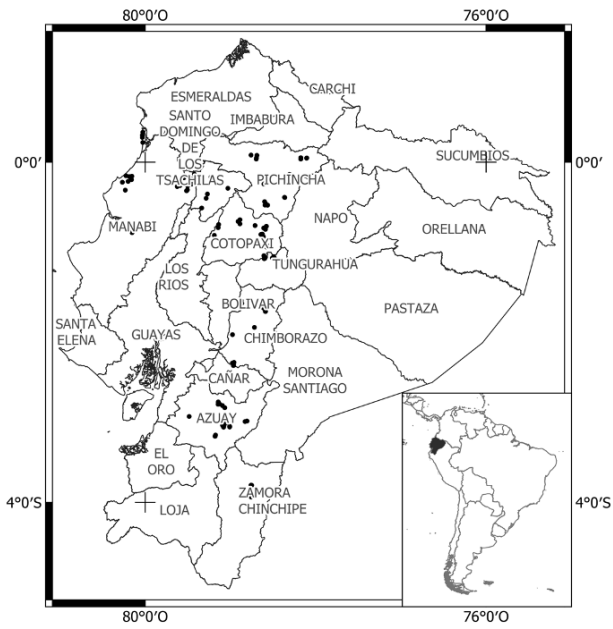
Figure 3. Spatial distribution of 139 bovine leukemia virus (BLV) cases (seroprevalence). Black dots indicate BLV seropositive cases.
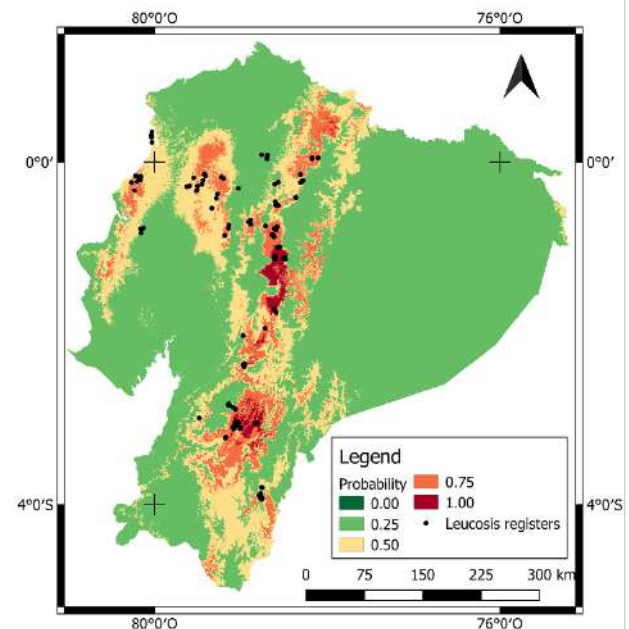
Figure 4. Probability map of bovine leukemia virus (BLV) seropositivity occurrence in Ecuador. The darkest colors show increased probability of seropositivity occurrence, while the black dots indicate seropositive of BLV cases.

Table 4. GEE model including the risk factors significantly (p<0.05) associated with bovine leukemia virus (BLV) seropositivity in dairy and dual-purpose herds from Ecuador (2008-2011).

Variables	Category	n	BLV seropositivity (%)	OR	95% CI
Sampling season	Wet	1,707	24.3	1.996	1.140-3.497
	Dry	961	13.4		
Artificial Insemination	Yes	674	33.4	2.215	1.402-3.501
	No	1,994	11.8		
Concrete floor	Yes	368	31.8	2.178	1.217-3.889
	No	2,300	15.0		
Presence of wild ruminants	Yes	439	29.6	2.998	1.788-5.027
	No	2,229	14.8		

OR: Odds Ratio; CI: Confidence interval.

*OR of the reference category was 1 in all cases. The QIC of the model is 2,218.

Discussion

This is the first nationwide survey to estimate the prevalence of antibodies and determine the risk factors associated with BLV seropositivity in Ecuador. Individual prevalence was 17.3%, agreeing with those previously reported in Brazil (7.6-50%) and Argentina (32.8%; Trono *et al.,* 2001; Amoril *et al.,* 2009; Rodriguez *et al.,* 2011). Herd prevalence was 37.8%, which is lower than that described in Argentina (84%; Trono *et al.,* 2001; Gutierrez *et al.,* 2011). Our results are consistent with the herd prevalence of BLV (34 to 94%) found in Colombia, Venezuela, Chile, Uruguay and US (Alfonso *et al.,* 1998; Rodriguez *et al.,* 2011; LaDronka *et al.,* 2018). However, herd prevalence could be underestimated because it is possible that herds with intra-herd prevalence lower than 35% were not detected as positive.

The results indicate that BLV infection is widespread among dairy and dual-purpose cattle herds in Ecuador. This may be due to poor management factors and the absence of eradication programs in this country. Another contributing factor for the high distribution of BLV infection could be the abundance of horseflies and blood-sucking insects present in the farms, especially in the warm wet season (Bech-Nielsen *et al.,* 1978; Chi *et al.,* 2002; Kobayashi *et al.,* 2014). Unfortunately, no routine control against horseflies is generally practiced in Ecuador.

Associations with BLV seropositivity only by univariable analysis (such as breed) or type of herd can be caused by other factors - not the variable itself, and have been included in the results section just as descriptive results. The GEE model included AI, concrete floor, presence of wild ruminants, and season of sampling. In Ecuador, AI (OR: 2.215; $C_{I95\%}$ = 1.402-3.501) usually implies rectal palpation (to test if the cow is already pregnant and if the reproductive tract is in good condition for an adequate pregnancy), which is usually performed without changing gloves and sleeves between animals. Although vertical transmission has been reported for leukosis virus, it is more probably that the high prevalence where AI is used be due to the mentioned inadequate practices. Our results are consistent with previous reports by Divers *et al.,* 1995; Rodriguez *et al.,* 2011; and Kobayashi *et al.,* 2014.

Concrete floor was also a risk factor for BLV seropositivity in the area (OR: 2.178; $CI_{95\%}$ = 1.217-3.889). This floor type is more abrasive than other floors in the area (e.g., pastures, ground, wood shaving), so animals could be at a higher risk of suffering wounds that would be a gateway for BLV. Therefore, cattle are enclosed, and contact with other animals is increased (Carbonero *et al.,* 2011; Saa *et al.,* 2012).

Improper handling practices, such as use of the same hypodermic needles for different

cows, can lead to iatrogenic transmission. These practices have been described as risk factors for other infections in Ecuador (Sargeant *et al.,* 1997; Saa *et al.,* 2012; Carbonero *et al.,* 2015). In addition, close contact during milking may favor BLV transmission.

Presence of wild ruminants in grazing areas was another next factor included in the model (OR: 2.998; $CI_{95\%}$ = 1.788-5.027). Presence of wild ruminants was considered positive (yes; 16.8%; 149/386) if the farmer affirmed that wild ruminants had been observed in the farm surroundings. Species belonging to Mazama and Odocoileus genus are the most common wild deer species in the area (Tirira, 2007). Chomel *et al.,* (1994) reported that black-tailed deer (Odocoileus hemionus) developed BLV infection under experimental conditions. However, Yokoi *et al.* (2009) did not find evidence of BLV infection in domestic sika deer (Cervus nipponyesoensis) in Japan. Although the role of wildlife, particularly wild ruminants, in BLV epidemiology remains unclear, this study suggests that they could play a role in the epidemiology of BLV infection. More studies are needed to clarify this point. Disease transmission between domestic and wild animals frequently results in severe consequences for the health of both species (Yokoi *et al.,* 2009; Martin *et al.,* 2011).

Sampling season (i.e., wet) was the other risk factor included in the model (OR: 1.996; $CI_{95\%}$ = 1.140-3.497). Other authors have described that BLV follows a seasonal pattern, and it is related to abundant horseflies and hematophagous insects during summer, being equivalent to the hot and wet season in the sampled areas of Ecuador. The role of different bloodsucking insects in the natural transmission of BLV is well known (Bech-Nielsen *et al.,* 1978; Hopkins *et al.,* 1997). In this sense, high presence of horseflies has been previously associated with BLV seropositivity (Kobayashi *et al.,* 2010; 2014). The fact that one of the frequent signs of BLV is lymphocytosis increases the probability of infection (Bech-Nielsen *et al.,* 1978).

In conclusion, these results indicate that BLV infection is widespread in dairy and dual-purpose cattle herds in Ecuador. The BLV seroprevalence is significantly influenced by AI, use of concrete floors, sampling season (wet), and presence of wild ruminants. Considering the risk factors determined by the model, our results suggests that a control program to fight against BLV infection should be implemented in Ecuador (Hopkins *et al.,* 1997).

Declarations

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Conflict of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

Author contributions

Luis Rodrigo Saa and Alfonso Carbonero participated in the design and coordination of the study, performed the data collection and statistical analyses, drafted the manuscript and performed the serological diagnoses. Luz María Castro, Lucía Guzmán and Natacha Fierro participated in the design of the study and reviewed the data analyses performed by Luis Rodrigo Saa, and Alfonso Carbonero. Fabián Reyes helped revise the data and helped draft the manuscript and maps. All of the authors read and approved the final manuscript.

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