Journal of Veterinary Medicine and
Animal Health

Full Length Research Paper

Seroprevalence and risk factors associated with bovine leukemia virus in Colombia

Diego Ortiz Ortega[1]*, Alfredo Sánchez[2], Julio Tobón[2], Yanira Chaparro[3], Sandra Cortés[3] and María Fernanda Gutiérrez[4]

[1]Corporación Colombiana de Investigación Agropecuaria – CORPOICA, Km 14 Vía Mosquera – Bogotá, Colombia.
[2]Empresa Colombiana de Productos Veterinarios – VECOL, Av. El dorado 82 – 93, Bogotá-Colombia.
[3]Laboratorio ZOOLAB, Carrera 73 No. 53-04 Bogotá – Colombia.
[4]Departamento de Microbiología, Pontificia Universidad Javeriana Cra 7 # 43 82 Ed 50, Bogotá – Colombia.

Received 20 January, 2016; Accepted 30 March, 2016

An epidemiological study was conducted to establish the prevalence of the Bovine Leukemia Virus (BLV) in Colombia and to describe risk and protecting factors associated with this infection disease. The study was performed with an observational descriptive cross-sectional process in twelve Colombian regions, by collecting blood samples from 8150 bovines in 390 cattle farms between February and September 2014. The seroprevalence obtained by enzyme-linked immunosorbent assay (ELISA) tests was 42.7% in animals and 67.7% in farms. The highest seroprevalence was found in Villavicencio with 91% in animals. The infection with blood parasites and another virus was attributed to be among the main risk factors associated to BLV. The use of individual needles during veterinary procedures was found to be the main source of protection against the virus. Climate data and ecological groups were recorded at sampling sites in order to elaborate geo-referencing maps by using analyzes of viral distribution around the country. Results obtained showed that there is a probability of an increase on the incidence of this pathology as well as a predictive issue associated with places and climate variables. It was found that developing epidemiological analyzes aiming to report and monitor the presence of this disease and its risk factors is the only alternative to generate prevention and control strategies.

Key words: Ecological groups, bovine leukemia virus (BLV), Colombia, enzootic bovine leucosis.

INTRODUCTION

Bovine leukemia is a neoplastic disease of cattle and is classified into enzootic and sporadic bovine leukosis. Enzootic bovine leucosis (EBL) is caused by bovine leukemia virus (BLV), which belongs to the genus delta retrovirus in the Retroviridae family. This disease is asymptomatic in 70% of infected cattle, it produces a persistent lymphocytosis in 30% and leukemia in 5% (Bartlett et al., 2014; Frie and Coussens, 2015).

Bovine leukemia virus does not exist as free viral particles in peripheral blood; rather than that, proviral

such as age, parity, and place.

Methodology

The frequency measurement obtained was the prevalence (OIE, 2008) only due to the lack of monitoring of the study population. This prevalence crossed with determinants that were established by formulating questions during surveys.

The analysis of the information collected in the field consisted of categorizing variables in a question form to be evaluated through listings, frequency and statistical tables. Listing information was stripped and a frequency analysis was developed over the refined database and obtaining absolute results and relative frequencies. The analysis indicated the number of cases followed by the cumulative percentage. The average, sum and standard deviation were presented if fields consisted of numerical values (Dean et al., 1991; Londoño 1996).

The ratio of animals and farms affected by BLV were exposed to a factor and compared with results of the same proportion not exposed to the factor. The results obtained were analytically processed in order to determine the association between clusters and to compare with referenced values from other publications. The variables from all categories were analyzed using the chi-square test (Martinez et al., 1997). Prevalence ratio (PR) was used to estimate the risk and the significance of the association between BLV, specific symptoms and a hypothetical causal factor (Dean et al., 1991). The PR was interpreted similarly to the relative risk (RR) which measures the association regardless of the used sampling method. The odds ratio (OR) was later used in the multivariate analysis (Martin et al., 1993).

A stratified analysis methodology was assessed to obtain free effect association measurements that can lead to confusing variables (Londoño, 1996; Martin et al., 1997). The methodology was also executed to study the interaction between variables, which were acting together on the observed effect (presence of BLV).

The OR was subsequently used in a multivariate analysis (Martin et al., 1997). The associated variables that showed significant numbers were also identified and multifactorial relationships were determined. The BLV in this analysis is explained by multiple factors or independent variables by using the basics of multivariate analysis through logistics regression methodology and also using the free software (Epi-Info 7) (Londoño 1996).

The quantitative variables consisted of verifying the homogeneity of variances in groups using the Bartlett test. After confirming the homogeneity of variances, the next step consisted of performing the traditional parametric analysis of variance (Fisher f Test). The Student t test was also used to compare adjacent averages.

The Kruskal-Wallis test was used in the case that there was no homogeneity between variances, and the media of each group were compared. The Mann-Whitney/Wilcoxon test was later used to assess the difference between adjacent media (Dean et al., 1992; Florez et al., 1994; Martinez et al., 1997).

The Ecological Niche models described by Carpenter et al. (1993) were used to predict the distribution of the BLV with the type of climate, and BIOCLIM (Bushby, 1991; Nix, 1986) or DOMAIN (Mekata et al., 2015) approaches were applied. The climate data was first taken at study Departments, geo-referencing maps were later elaborated including all 390 farms. Finally, the tools to predict the distribution of BLV were applied.

RESULTS

The assessment of the prevalence of BLV was conducted using samples of animals and farms. The seroprevalence in 12 surveyed regions was found to be 42.7% in cattle and 67.6% in farms. Table 1 shows the prevalence found in each Department and Municipalities. Villavicencio was found to be the place with higher seroprevalence in animals with 91.5%. El Rosal in Cundinamarca was found to have the lower seroprevalence with 19.5%. San Pedro de LosMilagros, as well as Aguachica and La Gloria, showed that 100% of their farms had, at least, one infected animal. The municipality of El Rosal was found to be the zone with the lowest percentage of infected farms with a 37%.

The analysis of risk factors was more focused on animals rather than on farms. The hypothesis testing was conducted to determine risk and protective factors related to the presence of BLV. The Chi^2 test was applied in order to obtain a PR. A risk factor was considered for PR values higher than 1 with an Low confidence Limit (LCL) greater than 1 and a p lower than 0.05. A protective factor was determined for PR values lower than 1 with a Upper Confidence Limit (UCL) and p lower than 1 and 0.05, respectively. PR value equal to 1 indicated that the factor was not associated.

Among all data collected from surveys, seventy factors were included as a potential risk or protective factors. Thirty out of the seventy were found to be risk factors, where the presence of blood parasites was found to be the major factor with a PR of 27.4 (p=0.00000). Table 2 displays the presence of non-lactating cows on the farm that showed the lower risk of acquiring BLV with a PR of 1.45% (p=0.009). On the other hand, seven protective factors were found, where the use of individual needles was the most effective with an RP of 0.049 (p=0.000000). The lowest protective factor was found to be the purchasing of dairy animals with a PR of 0.71 (p=0.028) (Table 3).

Figure 1 show the geographical distribution of BLV obtained on 8150 animals in 390 farms at seven Departments. The red and yellow dots are showing positive and negative results, respectively.

The Ecological Niche model was applied on a geo-referenced map of farms to predict the places that were most likely to acquire BLV. The data was estimated from different climate models and analysis modes provided by the Intergovernmental Panel on Climate Change Data Distribution Center (1999). The original data was interpolated into a grid of ten minutes. Figure 1 shows the positive and negative results in red and blue dots, respectively. Figure 2 indicates the places with the highest risk of developing BLV. Results show yellow and orange areas corresponding to excellent sites for the virus to be found if the climate behaved similarly to the currently reported. The dark green areas indicate sites of low probability of finding the BLV.

Figure 3 shows the relationship between climate factors such as precipitation, temperature, and presence or absence of BLV. The comparison of climate variables between positive and negative farms was conducted according

Table 1. Prevalence of BLV in cattle of 12 municipalities at seven Departments of Colombia. Data reported from 390 farms and 8150 animals.

Department	Municipality	No. of farms	BLV +	%Seropreva	#Cattle	BLV +	%Seropreva
Antioquia	San Pedro de los milagros	29	29	100	1004	540	53.9
Boyacá	Sotaquirá	65	49	75.4	1000	311	31.1
Cesar	Aguachica	19	19	100	642	501	78
	La gloria	1	1	100	35	27	77.1
	Rio de oro	8	8	100	289	231	79.9
Córdoba	Monteria	8	5	62.5	1001	51	5.1
Cundinamarca	El rosal	8	3	37.5	231	45	19.5
	Madrid	5	4	80	81	21	25.9
	Puerto salgar	14	14	100	1005	362	36
	Subachoque	53	35	66	824	206	25
Nariño	Guachucal	151	68	45	1038	274	26.4
Meta	Villavicencio	29	29	100	1000	910	91
Total	-	390	264	67.7	8150	3480	42.7

Table 2. Risk factors associated with BLV obtained in 390 farms located at 12 municipalities of six Colombian Departments, 2014. LCI (lower confidence interval) of 95%; UCI (upper confidence interval) of 95 %.

Risk factors	Prevalence ratio	LCI 95%	UCI95%	p
Hemoparasites	27.38	3.90	192.47	0.000000
Respiratory syncytial virus	7.65	5.70	10.26	0.000000
Heifer abortion	7.42	1.09	50.64	0.001882
Bovine Paratuberculosis	7.41	3.88	14.16	0.000000
Parainfluenza Virus	5.93	4.20	8.35	0.000000
The farm poultry	5.43	3.39	8.70	0.000000
Third trimester abortion	4.20	1.10	16.02	0.004232
Milking	3.98	1.93	8.18	0.000001
First trimester abortion	3.50	1.18	10.36	0.002956
Abortion normal	3.38	1.85	6.17	0.000001
Abortion third two of gestation	3.05	1.41	6.56	0.000291
Foreman vaccine	3.02	1.18	7.69	0.003061
Retained placenta	2.79	1.83	4.26	0.000000
Abortion in cows	2.69	1.59	4.55	0.000008
Joint injury or trauma	2.67	1.24	5.71	0.001657
Calving problems Dystocia	2.41	1.50	3.87	0.000019
Stillbirth	2.36	1.11	5.03	0.006243
Second trimester abortion	2.20	1.04	4.66	0.012305
Bovine mastitis	2.20	1.58	3.05	0.000000
Infectious bovine rhinotracheitis	2.17	1.69	2.91	0.000000
Abortion third three of gestation	2.13	1.06	4.28	0.009906
Bovine viral diarrhea	2.07	1.53	2.79	0.000073
Dead animals buried	1.87	1.38	2.53	0.000032
Buying breeding animal	1.76	1.15	2.69	0.002893
Abortion buried	1.69	1.17	2.44	0.002063
Presence of *Dyctiocaulus viviparus*	1.63	1.05	2.53	0.012080
Leptospirosis in cattle	1.61	1.21	2.15	0.001110
cattle with agalactia	1.52	1.10	2.09	0.005427
Diarrhea	1.47	1.08	1.99	0.008222
Empty cows	1.45	1.08	1.94	0.009546

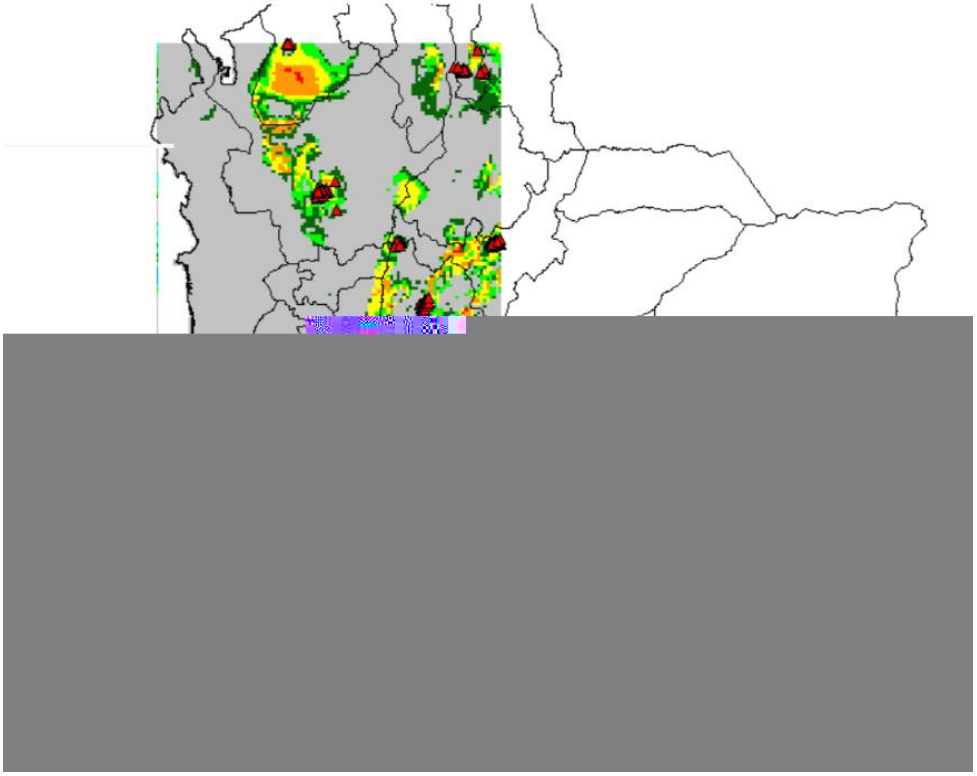

Figure 2. Predictive model of the presence of BLV. Red: BLV positive sites. Results show yellow and orange areas corresponding to excellent sites for the virus to be found if the climate behaved similarly to the currently reported. Dark and light green areas showed the low and medium probability of finding the BLV respectively.

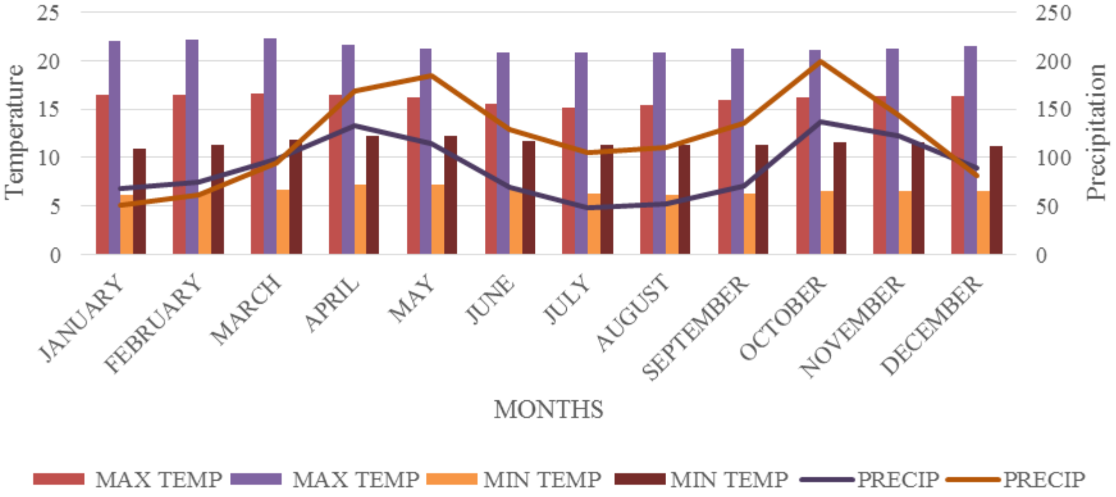

Figure 3. Comparison of climate variables between positive and negative farms. No significant differences were found between them. Blue bar: Maximum temperature in negative farms. Orange bars: Maximum temperature in positive farms. Gray bars: Minimum temperature in negative farms. Yellow bars: Minimum temperatures in positive farms. Blue line: Precipitation values in negative farms. Green line: Precipitation values in positive farms.

There is an important issue to be considered here. Most of the farms are very small, with an average number of cows of not more than 35 each. There are some exceptions, like the municipalities of Monteria and Puerto

Salgar, which have 125 and 72 animals on average, respectively. On the other end, we have Guachucal in Nariño, with only 7 cows on average. It supposes that small farms are handled carefully, then they have low risk to be infected. In bigger farms the risk increases because the care is not as cautious as it should be. It has been described that in larger herds, there might be an increased chance of animal exposure to the virus (Nekouei et al., 2015).

The first data to be analyzed is the high BLV prevalence of both, animals (42.7%) and farms (67.7%). The same analysis performed in Colombia in 2007 showed results between 34 and 50% per animal and farms, respectively, which means that the values increased and that the virus is present endemically with a considerable seroprevalence.

The seroprevalence is being compared with data from different countries such as Canada, which reported 45% for farms and around 10% for animals in 1980 and increased to 78% in 2015. Argentina and Japan reported 80% for animals in 1999 (Gutiérrez et al., 2015; Hernández-Herrera et al., 2011) and also United States presented 39% for animals and 83% for farms in 2007 (Bartlett et al., 2014; Frie and Coussens, 2015). Also, between 1997 and 2007, the prevalence of cattle had an average of 85% in the United States. These data show that there is a high and variable seroprevalence around the Americas and it is even higher when it is measured per farms rather than per animals. The referenced information apparently indicates that there are no tools to control the disease due to the lack of a real political will and a poor understanding of how this retrovirus can affect human population (Rodríguez et al., 2011).

Considering a lack of control and reduction strategies, an alternative solution is to avoid factors that cause the risk associated with the infection. The most relevant monitoring conditions are repeated infections because BLV-positive animals have some level of immuno-suppression. It means that they can easily get infected with a large number of etiology infection pathogens such as blood parasitic infections, viral diseases, bacterial diseases and lung parasites and pathogens as well as those associated with mastitis and abortions (Ohno et al., 2015; Rodríguez et al., 2011)

Therefore, considering these conditions as risk factors, the most important element will be to have a special monitoring of the animal with repeated infections as well as to prevent healthy animals from sharing the space, utensils or medical practices with them.

Risk factors obtained in this study are considered similar with those already described by several authors, such as the use of needles (one per animal), insemination or palpation gloves, the practice of insemination instead of using bulls to mount the female cattle, vector control for flies and birds, feeding with negative or heat-treated colostrum for BLV, selection and separation of seropositive animals from herd and the proper management and control of material used for practices such as vaccination, dehorning, insemination, etc (Bartlett et al., 2014; Hernández-Herrera et al., 2011). The use of pen for cows is perhaps the least risk factor reported by other authors. In Colombia, the pen is used for multiple activities such as surgeries, dehorning, insemination, delivery and management of sick animals making its use a risk factor for dissemination of the virus.

Two conditions are found as risk and protective factors; they consist of the person who applies vaccines and milk the animals on the farm. The vaccination executed by experts is considered as a protective factor, while the vaccination performed by any other worker on the farm is considered as a risk. Additionally, the mechanical milking became a risk factor compared to the manual milking which is considered a protective factor. These variables may be related to hygienic and public health standards and GMP (Handling and manufacturing best practices). Hand milking is a more hygienic practice while in the mechanical milking the virus can remain on the machine and potentially infect the next cow.

Several authors in the literature have reported some other alternatives to decrease the prevalence of BLV. They are summarized in the present study as the following: The use of single-use needles during vaccination or therapeutic protocols, the use of obstetric removable sleeves when used in one animal and other, the use of disposable materials (cleaning, disinfection or sterilization of reusable materials and surgical instruments at least) in procedures such as dehorning, tattooing, implantation, cauterization, castration or tagging the animal's ear, the use of electronic or gas combustion devices instead of surgery equipment during dehorning, the elimination of insects, particularly in densely populated agricultural areas (milking areas, stalls and barns), in order to minimize possible transmission between cattle through arthropod vectors, direct mounting artificial insemination and embryo transfer with BLV-free donors and bulls (Rodríguez et al., 2011). Supplying foods such as carrots and flour is also considered a protective factor because they are supplies that reinforce the diet at a time when food decreases and improve the nutritional level of animals.

Among the other protective factors, the drinking water is also included. In Colombia, it is usual to give potable water to the cows. The same water used for human consumption. This water is free of pathogens and any kind of substances that can cause disease. Selling animals for release is a protective factor because they are usually led to the slaughterhouses to be sold as meat. In this case, the virus cycle is interrupted and it stops its infection to more animals.

Having high seroprevalence of BLV in animals and farms, a distribution of the virus in cattle, losses to livestock in our country and the possibility of a zoonotic infection, indicate the importance of developing more studies to monitor the virus. They should also generate preventive policies that will reduce the virus presence to prevent future problems that could have humans infected with BLV.

Climatic factors such as precipitation and temperature constitute determining factors in the occurrence of the disease. Results have shown significant differences between positive and negative farms ($p < 0.05$). A high rainfall is reported in negative farms and a lower rainfall in positive farms during December, January, February and March. The rainfall results changed during the rest of the year indicating high rainfall in positive farms as compared with low rainfall in negative farms promoting the positivity. The maximum and minimum temperatures were higher in positive farms as compared with negative farms throughout the year.

These results become an important decision-making tool for prevention and control. They allow having an adequate resource distribution to generate tool intervention programs. While the results are similar to those reported by other authors around the word, avoiding the risk and implementing protective factors are initiatives linked to the cultural process. For example, the use of poultry in Colombia is implemented not only to isolate animals but also with iatrogenic objective, for this reason good hand hygiene standards must be implemented in order to reduce the risk that means using contaminated pens.

Nevertheless, some reports already showed the presence of antibodies and viral genome segments in humans, specifically in women with breast cancer (Buehring et al., 2003; Gutiérrez et al., 2015; Anonymous, 2007; Ochoa-Cruz et al., 2006; Schwartz et al., 1997). The information suggests the presence of the retrovirus in humans; however, the authors have not referenced the statement as a zoonotic disease. Despite our conclusion, it is important to continue developing epidemiological analyzes tending to report and monitor the presence of this disease and its risk factors; this is the only alternative to generate prevention and control strategies.

CONCLUSION AND RECOMMENDATIONS

The results of the research show seroprevalence of 42.7% in animals and 67.7% in farms. The infection with blood parasites and another virus was attributed to be among the main risk factors associated to BLV. The use of individual needles during veterinary procedures was found to be the main source of protection against the virus. EBL should be included in the group of diseases categorized as mandatory notifiable in Colombia.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors would like to thank Dra. Katia Benavidez from Universidad de Nariño, ProyGuachucal; Dra. Clemencia Fandiño and coworkers from Universidad del Tolima, Proy Puerto Salgar; Dr. Agustín Góngora and coworkers from Universidad de los Llanos, Proy Villavicencio; Dr Darío Cárdenas and coworkers from Universidad Cooperativa de Colombia, Proy Villavicencio; Dr. Martin Pulido and coworkers from UPTC, Proy Sotaquirá; Dr. Salim Mathar and coworkers from Universidad de Córdoba, Proyecto Montería; and Dra. Marta Olivera and coworkers from Universidad de Antioquia, Proy San Pedro de los Milagros.

REFERENCES

Anonymous (2007). Prevalencia de cáncer de mama en America Latina. Salud: Mujer Saludable. http://www.deguate.com/salud/article_5127.shtml.

Bartlett P, Sordillo L, Byrem T, Norby B, Grooms D, Swenson C, Zalucha J, Erskine R (2014). Options for the control of bovine leukemia virus in dairy cattle. JAVMA 244(8):914-922.

Buehring G, Philpott S, Yeon Choi K (2003). Humans have antidodies reactive with Bovine leukemia Virus. AIDS Res. Hum. Retrovirus 19(12):1105-1113.

Bushby J (1991). BIOCLIM - A bioclimate analysis and prediction system. Plant Prot. Quart. 6:8-9.

Carpenter G, Gillison A, Winter J (1993). DOMAIN: a flexible modelling procedure for mapping potential distributions of plants and animals. Biodivers. Conserv. 2:667-680.

Dean A, Dean J, Buron A, Dicker R (1992). Epi Info, versión 5. Epidemiología con microordenadores. Division of Surveillance and Epidemiology. Epidemiology Program Office Center for Disease Control Atlanta, Georgia 30333 USA .

Florez T, Gonzalez E, Hernandez Z, Herrera V, Londoño F, Lopez H, Mazuera M, Mejia V, Ramirez C, Rojas L (1994). Curso Modular de Epidemiología Básica. Universidad de Antioquia. Facultad Nacional de salud Pública "Héctor Abad Gómez". Organización Panamericana de la Salud Segunda edición. Editores Flórez y Mazuera. Medellín Colombia.

Frie M, Coussens P (2015). Bovine leukemia virus: A major silent threat to proper immune responses in cattle. Vet. Immunol. Immunopathol. 163(3-4):103-114.

Gutiérrez G, Lomonaco M, Alvarez I, Fernandez F, Trono K (2015). Characterization of colostrum from dams of BLV endemic dairy herds. Vet. Microbiol. 177:366-369.

Hernández-Herrera D, Posso-Terranova A, Benavides JA, Muñoz-Flórez J, Giovambattista G, Álvarez-Franco L (2011). Detección del Virus de la leucemia Bovina en ganado criollo colombiano mediante PCR anidada. Acta Agronómica 60(4):312-318.

Kobayashi S, Hidano A, Tsutsui T, Yamamoto T, Hayama Y, Nishida T, Muroga N, Konishi M, Kameyama K, Murakami K (2014). Analysis of risk factors associated with bovine leukemia virus seropositivity within dairy and beef breeding farms in Japan: A nationwide survey. Res. Vet. Sci. 96:47-53.

Londoño F (1996). Metodología de la Investigación Epidemiológica. Editorial Universidad de Antioquia YULUKA Salud Pública Primera reimpresión Medellín (Colombia)

Martin S, Meek A, Willebreg P (1997). Epidemiología Veterinaria. Principios y Métodos. (Editorial Acribia S.A. Zaragoza (España).):384.

Martinez B, Martinez R (1997). Diseño de Experimentos. Análisis de Datos Estándar y no Estándar (Fondo Nacional Universitario. Editorial Guadalupe Ltda. Bogotá, Colombia):479

Murakami K, Kobayashi S, Konishi M, Kameyama K-i, Yamamoto T, Tsutsui T (2011). The recent prevalence of bovine leukemia virus (BLV) infection among Japanese cattle. Vet. Microbiol. 148(1):84-88.

Nekouei O, Van Leeuwena J, Sanchez J, Keltonb D, Tiwari A, Keefea G (2015). Herd-level risk factors for infection with bovine leukemia virus in Canadian dairy herds. Preventive Vet. Med. 119:105-113.

Nix H (1986). A biogeographic analysis of Australian elapid snakes. in R Longmore (ed)Atlas of Elapid Snakes of AustraliaAustralian Flora and Fauna 7:415.

Ochoa-Cruz A, Uribe A, Gutierrez M (2006). Estudio del potencial zoonótico del virus de la leucosis bovina y su presencia en casos de cáncer de seno. Universitas Scientiarum 11(2):31-40.

Ohno A, Takeshima S, Matsumoto Y, Aida Y (2015). Risk factors associated with increased bovine leukemia virus proviral load in infected cattle in Japan from 2012 to 2014. Virus Res. 210:283-290.

Rodríguez S, Florins A, Gillet N, Rrogniez A, Sánchez-Alcaraz M, Boxus M, Boulanger F, Gutiérrez G, Trono K, Alvarez I (2011). Preventive and therapeutic strategies for bovine leukemia virus: lessons for HTLV. Viruses 3:1210-1248.

Schwartz I, Chevallier N, Belloc C, Le Rhun D, Lainé V, Berthelmy M, Mateo A, Levy D (1997). Bovine leukaemia virus- induced lymphocytosis in sheep is associated with reduction of spontaneous B cell apoptosis. J. Gen. Virol. 78:153- 162.

Şevik M, Avcı O, İnce O (2015). An 8-year longitudinal sero-epidemiological study of bovine leukaemia virus (BLV) infection in dairy cattle in Turkey and analysis of risk factors associated with BLV seropositivity. Trop. Anim. Health Prod 47:715-720.

Thrusfield M (2005). Veterinary Epidemiology Third Edition (Edit Blackwell Publishing).