Scientific paper / Artículo científico

AGRICULTURAL SCIENCES

LA GRANJA: REVISTA DE CIENCIAS DE LA VIDA

pISSN:1390-3799; eISSN:1390-8596

http://doi.org/10.17163/lgr.n26.2017.11

SEROPREVALENCE OF BOVINE ENZOTIC LEUCOSIS IN 6 TO 24 MONTHS ANIMALS, IN MANABI, PICHINCHA AND CHIMBORAZO, ECUADOR

SEROPREVALENCIA DE LEUCOSIS ENZOÓTICA BOVINA EN ANIMALES ENTRE 6 A 24 MESES EN LAS PROVINCIAS DE MANABÍ, PICHINCHA Y CHIMBORAZO - ECUADOR

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Manuscript submitted on June 13, 2017. Accepted, after review, on August 22, 2017. Published on September 1, 2017.

Abstract

Enzootic Bovine Leukemia is a viral disease that can manifest itself by the presence of tumors or asymptomatically in cattle of any age category. Its socioeconomic significance lies in the decline in milk productivity, reproductive losses, forfeitures in abattoirs, and prevents the export of livestock or derivatives. The objective of this research was to determine the seroprevalence of viral enzootic bovine on animals under two years of Pichincha, ManabÃ and Chimborazo, provinces that represent important sources of milk production. There were 3 307 samples of bovine blood serum analyzed, obtained from National sampling of Aphtose Fever organized by AGROCALIDAD. Samples were analyzed by serological technique indirect ELISA, 480 samples of Pichincha, 2 348 of ManabÃ and 479 Chimborazo. Pichincha province presented 8.13% seroprevalence, with the highest percentage of sick animals in Mejia, one of EcuadorÂŽs main dairy basin. It was observed 0.89% seroprevalence of Manabi and Chimborazo 3.13%. The presence of the disease was also assessed according to the variables: climate, altitude, age and purpose of cattle, using chi squared to determine the relationship between them and the appearance of BVL. The investigation showed no association, showing this result of 105.14, 7.48, 38.87, 140.91 against p values 11.34, 9.21, 18.48 and 9.21 about weather, age, altitude and purpose, respectively. A greater tendency to get sick was revealed in temperate climates and higher altitudes, which could be explained by the similarity in practices of intensive exploitation. From the data obtained, further research can be made, because it is a study base line that can lead to develop a sanitary control plan of BVL from an early age of cattle.

Keywords: Seroprevalence, oncogenic, leukosis, leukemia, climate, altitude, purpose, age.

Resumen

La Leucosis Enzoótica Bovina (LEB o LVB) es una enfermedad vírica, manifestada por la presencia de neoplasias y también presentándose de forma asintomática en bovinos de cualquier edad. Su trascendencia socioeconómica radica en las pérdidas por disminución de la productividad lechera, pérdidas reproductivas y decomisos en camales, impidiendo también la exportación de ganado o derivados del mismo. El objetivo de esta investigación fue determinar la seroprevalencia de LEB en animales menores de dos años en las provincias de Pichincha, Manabí y Chimborazo, las cuales reportan gran producción lechera en Ecuador. Se analizaron 3 307 muestras de suero sanguíneo bovino mediante la técnica serológica ELISA indirecto, de las cuales 480 fueron de Pichincha, 2 348 de Manabí y 479 de Chimborazo. Pichincha presentó una seroprevalencia de 8.13%, con el mayor porcentaje de animales enfermos en la principal cuenca lechera del país, el cantón Mejía. En Manabí se observó 0.89% de seroprevalencia de LEB y en Chimborazo 3.13%. Utilizando chi cuadrado para determinar la relación entre las variables evaluadas y la aparición de LEB, se reveló que no existe asociación, mostrando un resultado de 105.14, 7.48, 140.91 y 38.87 frente a valores de p de 11.34 (clima), 9.21 (altitud), 18.48 (edad) y 9.21 (propósito del ganado). Sin embargo, se observó mayor tendencia a enfermar en clima templado y mayor altitud, lo que pudo explicarse por la similitud en las prácticas de manejo y la explotación tipo intensivo. Los datos obtenidos significan una línea base de estudio de la cual se puede partir para elaborar un plan de control sanitario de LEB desde edades tempranas del ganado bovino y emprender en otras investigaciones.

Palabras claves: Seroprevalencia, oncogénica, Leucosis bovina, clima, altitud, propósito, edad.

Suggested Citation: Vásconez-Hernández, A., Sandoval-Valencia, P., Puga-Torres, B., Cueva-Jácome, F. 2017. Seroprevalence of bovine enzotic leucosis in 6 to 24 months animals, in Manabi, Pichincha and Chimborazo, Ecuador. La Granja: Journal of Life Sciences. Vol. 26(2):131-141. pISSN:1390-3799; eISSN:1390-8596.

1 Introduction

Bovine Enzootic Leukosis (LEB), also known as Bovine Viral Leukosis (LVB), is a persistent infectious disease caused by a retrovirus closely related to human T-lymphotropic virus type 1 (HTLV-1) (Rodríguez et al., 2011) which mainly affects lymphocytes B (Algorta et al., 2014), causing an increase in its circulating percentage, the accumulation of which can determine the appearance of neoplasms, and can be disseminated in different organs (Radostits, 2000) . The forms of manifestation observed include the presence of tumors or persistent lymphocytosis (sustained increase of blood lymphocytes) (OIE-World Organization for Animal Health- 2011).

The major problem is that a large percentage of the infected animals are asymptomatic, being the main cause of contagion, and therefore little control in the mobilization of cattle, So it is essential to monitor the health of animals through serological tests (Gatti Assandri, 2008, OIE, 2011), as shown in Japan, it can lead to multiple invasions of genetically different strains of LEB (Matsumura et al., 2011). It has even been indicated that the LEB virus could be associated with breast cancer in humans, since the virus has been found in people with this disease (Ochoa et al., 2006).

All bovine breeds, at any age, are susceptible to disease, with this characteristic being observed more frequently in dairy cattle compared to beef cattle (Giraudo et al., 2010). The most frequent form of contagion is horizontal transmission, through contact with contaminated body fluids through iatrogenic way (Gatti Assandri, 2008). Hematophagous insects act as vectors of the disease, and although vertical, intrauterine or calostral transmission (OIE, 2011) is not very common, it could cause a higher percentage of diseased animals in an infected farm, with consequent economic losses for the producer. In studies performed in Korea on 30 animals suffering from LEB, neoplastic tissues were observed in the lymph nodes of all animals; Nodules in the intestine (96.4%), heart (88.9%), stomach (73.1%) and diaphragm (62.5%) were also observed, suggesting metastasis of the disease (Yoon et al. , 2005). Therefore, greater control of LEB is necessary in order to monitor and follow up the animals, especially in the younger ones, since they represent a greater risk for production.

In Ecuador, it is not very well known pathology among cattle ranchers. Therefore, despite its health importance, prevention and control measures, transmission routes and information about its effects on production have not been widely disseminated; additionally, there is little accurate data on the current prevalence of LEB in the country. The aim is to take care of the quality of production to offer locally safe food and with the same requirements of the products of export, establishing control measures against several diseases that reduce the production and limit the reach to the international market. One of these diseases is LEB, not only because of its health importance but also because of the economic implications, since it significantly reduces dairy production and the birth of healthy offspring and causes a drop in immunity, making affected animals more likely to acquire other diseases (Chamizo Pestana, 2005), causing significant seizures and altering the quality of the meat, which impairs the profitability of the herdsman and hinders the export possibilities (Betancur and Rodas, 2008).

For these reasons the objective of our investigation was to determine the seroprevalence of LEB in young animals from the provinces of Pichincha, Manabí and Chimborazo (the largest dairy in Ecuador) to know their health status in relation to this disease and to establish early sanitary control measures, generating a base line of study that allows Ecuador to carry out a program of control and eradication of the disease by the Ecuadorian Agency for Quality Assurance of Agro (AGROCALIDAD).

2 Methodology

2.1 Study population

The study population was bovine from six to twenty four months old, from three of the provinces with the highest milk production. A total of 3 307 samples of bovine blood serum, obtained by stratified random selection in the Ecuadorian Sampling of FMD carried out in 2014 (Table 1), were used. Of the total samples, 480 corresponded to Pichincha (located in the north of the country and in the inter-Andean region); 479 to Chimborazo (south of the country and in the inter-Andean region) and 2 348 to Manabí (located to the west of the country and in the coastal region).

Table 1. Number of samples, by province and by canton

Province	**Canton**	**N° of samples**	**Total**
Manabí	Portoviejo	20	2348
	Bolívar	12	
	Chone	662	
	El Carmen	482	
	Flavio Alfaro	346	
	Jama	25	
	Jipijapa	10	
	Paján	148	
	Pedernales	291	
	Pichincha	141	
	San Vicente	108	
	Santa Ana	38	
	Sucre	55	
	Tosagua	10	
Chimborazo	Riobamba	71	479
	Alausí	110	
	Chambo	36	
	Chunchi	20	
	Colta	99	
	Cumandá	11	
	Guamote	42	
	Guano	80	
	Pallatanga	10	
Pichincha	Cayambe	30	480
	Mejía	126	
	Pedro Moncayo	16	
	Pedro Vicente Maldonado	55	
	Puerto Quito	55	
	Quito	105	
	Rumiñahui	48	
	San Miguel de los Bancos	45	

2.2 Research design

Descriptive study of the finding of LEB in young animals, manifested by the presence of antibodies in serum samples of these animals. For this reason it is an observational study because the variables under consideration were not modified, but the obtained results were used directly for analysis and discussion. The 3 307 bovine serum samples were analyzed using the indirect ELISA serology technique.

2.2.1 Sample selection

With the National Foot-and-Mouth Disease Sampling database, we verified the entrance code of each sample, confirming its existence, origin and relevant data for the investigation. The working protocols were developed for each ELISA microplate, using 40 of them.

2.2.2 Sample Processing

The instructions and recommendations of the SVANOVA kit manufacturer (2015) were followed for both sample processing and storage. ELISA microplates, reagents and samples were set (for 20 minutes). The ELISA plate was loaded with 100 ul of diluent in each of the 96 bowls; depositing 4 ul in the first four bowls, positive and negative controls in duplicate and from the fifth well the samples under study. Once loaded, the adhesive paper was covered to avoid contamination during incubation and stirring for one hour at 37 ° C. The wash solution was prepared (PBS + Tween20 solution) in 1:20 concentration. The contents of the wells were washed (3 times) with 300 ul of the prepared solution. In each well we placed 100 ÎŒl of Conjugate Solution, to then seal the plate with the same adhesive film, allowing the plate to incubate for 1 hour at 37 ° C. Subsequently we performed a last wash, after which we placed 100 ul of Substrate Solution in each well, which after 15 minutes of incubation in darkness revealed the coloration. To conclude, 50 ul of Brake solution was added to each well to pass the plate through the XChek ELISA Plate Reader, which is a spectrophotometer that measures the optical density at 450 nm (wavelength).

3 Results

The total apparent seroprevalence found in the 3 307 bovine samples, between 6 and 24 months of age in the three study provinces, was 2.27%; Whereas the real seroprevalence, taking into account the data of sensitivity and specificity of the kit (98 - 100%) is 2.07% (Table 2). We found seroprevalences of 0.89% in Manabí, 3.13% in Chimborazo and 8.13% in Pichincha (Table 2).

For teaching purposes, the following categories were classified as follows: Highlands from 2 to 10 ° C, temperate from 11 to 18 ° C, subtropical from 19 to 23 ° C and tropical from 24 to 30 ° C (Table 3).

3.1 Presence of LEB according to age

In order to quantify the percentage of LEB in young animals from the provinces with the highest dairy production, cattle were categorized from 6 to 12 months, from 13 to 18 and from 19 to 24 months (Table 4).

3.2 Presence of LEB according to altitude

For teaching purposes, this variable was categorized from 0 (zero) meters above sea level (500 ms) to 500, from 501 to 1000 masl and successively ascending in groups of 500 masl (Table 5).

3.3 Presence of LEB according to the purpose of cattle

According to the classification of milk, meat and double purpose, it was found that in the studied provinces, the highest percentage of animals positive for LEB occurs in milk animals (4.47%), followed by 3.52% Cattle for meat and 1.02% for double purpose cattle (Table 6). Taking into account only the two provinces of the Sierra (Highlands), in 580 animals destined to milk production was found a 7.07% presence of LEB in 53 animals corresponding to beef cattle, 5.66% of positive animals and 3.07% in 326 animals sampled as mixed cattle.

4 Presence of LEB according to the provinces and cantons

The 3 307 analyzed samples belong to 268 sites, of which 100 were from Chimborazo, 131 from Mana-

Table 2. Apparent seroprevalence of LEB in young animals from the provinces with the largest milk production in Ecuador

Province	Total	Positive		Negative		Prevalence Apparent %	Prevalence Real %
		Number	%	Number	%		
Chimborazo	479	15	3,13	464	96,87	3,13	2.16
Pichincha	480	39	8,13	441	91,86	8,13	7.20
Manabí	2348	21	0,89	2327	99,11	0,89	<0.024*
Total	**3307**	**75**	**2.26**	**3232**	**97.73**	**2.27**	**2.07**

*Seroprevalence real between (-0.24 % and 0.024 %)

Table 3. Percentage of LEB presence according to climate in Chimborazo, Pichincha and Manabí

Climate	Number / %	Positive	Negative	Presence %	Total
Páramo	**Number**	8	245	3,16	253
	%	3,16	96,84		
Templado	**Number**	42	458	8,4	500
	%	8,4	91,6		
Subtropical	**Number**	9	715	1,24	724
	%	1,24	98,75		
Tropical	**Number**	16	1814	0,87	1830
	%	0,87	99,13		

Table 4. Presence of LEB in young animals from the provinces with the highest milk production in Ecuador, according to age

Age (Month)	Number / %	Positive	Negative	Presence %	Total
6 a 12	**Number**	30	1735	1,7	1 765
	%	1,7	98,3		
13 a 18	**Number**	35	1035	3,27	1 070
	%	3,27	96,73		
19 a 24	**Number**	10	462	2,12	472
	%	2,12	97,88		

Table 5. Presence of LEB in young animals from the provinces with the highest milk production in Ecuador, according to altitude

Altitude	Number / %	Positive	Negative	Presence %	Total
0 a 500	**Number**	23	2 391	0,95	2 414
	%	0,95	99,05		
501 a 1 000	**Number**	1	64	1,54	65
	%	1,54	98,46		
1 001 a 1 500	**Number**	3	110	2,65	113
	%	2,65	97,35		
1 501 a 2 000	**Number**	3	16	15,79	19
	%	15,79	84,21		
2 001 a 2 500	**Number**	1	79	1,25	80
	%	1,25	98,75		
2 501 a 3 000	**Number**	38	335	10,19	373
	%	10,19	89,81		
3 001 a 3 500	**Number**	6	220	2,65	226
	%	2,65	97,35		
3 501 a 4 000	**Number**	0	17	0	17
	%	0	100		

Table 6. Presence of LEB in young animals of the provinces with the largest dairy production in Ecuador, according to purpose

Purpose	Number / %	Positive	Negative	Presence %	Total
Milk	**Number**	47	1 004	4,47	1 051
	%	4,47	95,53		
Mixed	**Number**	21	2036	1,02	2 057
	%	1,02	98,98		
Meat	**Number**	7	192	3,52	199
	%	3,52	96,48		

Table 7. Percentage of disease presence in cantons of Chimborazo, Pichincha and Manabí

Province	Canton	Number/ %	Positive	Negative	Presence %	Total
Chimborazo	Pallatanga	**Number**	0	10	0	10
		%	0	100		
	Chambo	**Number**	6	30	16,67	36
		%	16,67	83,33		
	Chunchi	**Number**	0	20	0	20
		%	0	100		
	Riobamba	**Number**	1	70	1,41	71
		%	1,41	98,59		
	Guamote	**Number**	2	40	4,76	42
		%	4,76	95,24		
	Colta	**Number**	0	99	0	99
		%	0	100		
	Alausí	**Number**	2	108	1,82	110
		%	1,82	98,18		
	Guano	**Number**	3	77	3,75	80
		%	3,75	96,25		
	Cumandá	**Number**	1	10	9,09	11
		%	9,09	90,91		
Pichincha	Pedro Moncayo	**Number**	0	16	0	16
		%	0	100		
	Rumiñahui	**Number**	2	46	4,17	48
		%	4,17	95,83		
	Puerto Quito	**Number**	1	54	1,82	55
		%	1,82	98,18		
	Quito	**Number**	6	99	5,71	105
		%	5,71	94,29		
	Mejía	**Number**	26	100	20,63	126
		%	20,63	79,37		
	Cayambe	**Number**	3	27	10	30
		%	10	90		
	Pedro Vicente Maldonado	**Number**	1	54	1,82	55
		%	1,82	98,18		
	San Miguel de los Bancos	**Number**	0	45	0	45
Manabí	Jipijapa	**Number**	0	10	0	10
		%	0	100		
	Jama	**Number**	0	25	0	25
		%	0	100		
	Flavio Alfaro	**Number**	3	343	0,87	346
		%	0,87	99,13		
	El Carmen	**Number**	4	478	0,83	482
		%	0,83	99,17		
	Sucre	**Number**	0	55	0	55
		%	0	100		
	Paján	**Number**	7	141	4,73	148
		%	4,73	95,27		
	Santa Ana	**Number**	0	38	0	38
		%	0	100		
	Pedernales	**Number**	0	291	0	291
		%	0	100		
	Tosagua	**Number**	0	10	0	10
		%	0	100		
	Portoviejo	**Number**	0	20	0	20
		%	0	100		
	Bolívar	**Number**	1	11	8,33	12
		%	8,33	91,67		
	Chone	**Number**	5	657	0,76	662
		%	0,76	99,24		
	Pichincha	**Number**	0	141	0	141
		%	0	100		
	San Vicente	**Number**	1	107	0,93	108

bí and 37 from Pichincha (Table 7). In Chimborazo 6 LEB positive farms (6%) were found, in Manabí 13 (9.92%) and in Pichincha 15 positive farms (40.54%).

Regarding the sex of the animals, it was found that of the 75 positive animals, 58 were females (77.33%) and 17 males (22.66%).

In the main milk basin of Ecuador, the Mejía canton of the province of Pichincha, an alarming percentage of LEB (20.63%) was found, possibly due to the intensive production systems that allow greater contact between animals and humans. Some of the possible causes can be attributed to stress and inefficient biosecurity practices, leading to a greater spread of disease among animals.

4.1 Statistical Analysis

The general chi-square statistical analysis, using a 1% probability for each variable, shows that there is a highly significant dependence between climate, altitude and purpose with the appearance of Bovine Enzootic Leucosis in animals between 6 and 24 months. The age variable is not determinant for the finding of the disease. However, in the same analysis by each province, it was evidenced that in Chimborazo and Manabí there is no dependence between the disease and the variables, whereas in Pichincha a highly significant dependence was found between the finding of the disease and the climate variables and altitude (Table 8).

5 Discussion

The results indicate that there is no significant relationship between the variables and the occurrence of LEB, but there is a dependence of the disease on the climate and altitude, since there is a great difference of values between the province of the coast (Manabí) and The inter-Andean region (Pichincha and Chimborazo), indicating a tendency to present more cases of LEB in dairy cattle. However, this trend could be explained by the differences between management practices in dairy and beef cattle, or by the type of farm, since it is customary to raise beef cattle in extensive farms where there is no greater contact and Sanitary and nutritional management of animals. Similar results were reported by Delgado et al. (2009), which shows the high relationship between the occurrence of LEB and the type of exploitation, emphasizing the importance of the human factor, because intensive management practices allow a higher rate of contagion, a fact that is reaffirmed by Bautista Et al. (2008) who in their seroprevalence studies in several departments of Colombia report the high significance in the association with the type of farms and the similarity of livestock practices in animals destined to milk production in cold weather with findings of higher prevalences than in beef cattle.

In this research, the relationship between sick animals and age is not perceptible, with very close percentages being observed, which coincides with a great deal of LEB research, since the ages under study range from three years onwards. Puma and Yanza (2013) in their study in 3 parishes of the canton Paute (Azuay-Ecuador) report a 6.1% seroprevalence of LEB, Bonifaz and Ulcuango (2015) in the Santo Domingo Community 1 of the Ayora Parish of Cayambe Canton (Pichincha -Ecuador) found a prevalence of infection of 5.6% of a total of 250 samples, while Torres and Jaramillo (2001), in their work in several parishes of Cuenca (Ecuador), report seroprevalences in animals of 4 to 6 Years of 20.2%, from 7 to 9 years of 22.22%, from 10 to 12 years old 25% and over 12 years of age a seroprevalence of 50%, in this study we observed a proportional increase between the age of the animals and the number of sick animals, is largely due to the fact that adult animals with greater permanence in the herd are more likely to be infected, which is also mentioned in the study by Carrero et al. (2008) in Colombia, where animals from 6 months of age were taken into account, but with impressive results, where 54% of animals from 6 months to 1 years were positive for LEB, animals aged 1 to 2 years 62% Of 2 to 3 years 75% and over 3 years the seroprevalence was 87%. Similarly, in studies conducted in 7 prefectures of Japan (Murakami et al., 2011), the overall prevalence of LEB infection was 28.6%, of which dairy cattle (34.7%) were higher than in Fattening cattle (7.9%) and breeding cattle (16.3%). It is interesting to mention this research, because it is a border country and represents a potential threat, whether by cattle passage or possibly vectors. In the same study it was concluded that vectors (mosquitoes and ticks) play an important route of contagion, but in Ecuador this theory is not supported since the results in subtropical and tropical climate are really low. It is also important to mention the study of Vallejo (1991) as one of the most extensive, where a

Table 8. Overall Chi square result (Pichincha, Manabí, Chimborazo)

Variable	Value	Degrees of freedom (d.f)	Probability (p %)
Clima	105.14	3	11.34
Edad	7.48	2	9.21
Altitud	140.91	7	18.48
Propósito	38.87	2	9.21

prevalence of 14.3% of LEB was found in farms on the Ecuadorian coast (Guayas, El Oro, Manabí, Esmeraldas) but a large part of the animals came from Costa Rica; without these animals, the positive population was showing values up to 5% prevalence. This denotes the importance of a quarantine monitoring and control of the animals that arrive at a Productive Unit and the respective record. In this investigation, 5 of the 75 positive animals that were acquired were found which, if not examined in time, represent a threat of being the focus of virus spread in a free or controlled farm. Our results do not coincide with those provided by Algorta et al. (2014), who in the south of Uruguay found prevalence between 11 and 75%. Likewise, in samples of bovine semen, Ali et al. (2011) found a prevalence of 20.93% of the total samples.

When comparing this data with the research in Manabí of Zambrano and Burgos (2010), it is observed that a large number of calves (88.30%) and bulls (10.64%) Reacted positively to the test, and that 24.52% of calves and 66.26% of cows were positive. Likewise, it is mentioned that cows in production represent the highest percentage of diseased animals and are also a source of contagion for younger animals as evidenced in this research and are even a source of contagion through colostrum to calves with milk of seropositive Cows. In relation to the study by Cadavid in Colombia (2012), it is exposed that 5% of the annual production of positive cows is lost and this represents twice that of the OIE (2.5%) added to 1.3% of discard.

Acknowledgment

To the Ecuadorian Agency of Quality Assurance of Agro (AGROCALIDAD) who supported directly in this project, facilitating its infrastructure, technology and chemical and biological reagents.

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