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Seroprevalence Against Bovine Leukaemia Virus in Dairy Cattle in Bolivia

[1]R. López, [2]K. Suzuki, [2]E.T. González, [1]G. Ruiz, [1]A. Loza,
[1]C. Coca, [1]J.A.C. Pereira and [2]M.R. Pecoraro
[1]Facultad de Ciencias Veterinarias, Universidad Autónoma Gabriel René Moreno, Santa Cruz de la Sierra, Bolivia
[2]Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata (FCV-UNLP), La Plata, Argentina

Abstract: This study determined seroprevalence against bovine leukaemia virus and investigated the risk factors with the seropositivity of the virus in dairy cattle in Bolivia. Ninety-eight farms in five different provinces in Santa Cruz Department were visited to study 1823 dairy cattle. Questionnaire interviews, blood sampling and inspection of skin were performed at each study farm. Individual-cattle sera were analysed using the agarose gel immunodiffusion (AGID) diagnostic method for the detection of antibody against bovine leukaemia virus. The overall percentage of test positive against bovine leukaemia virus was 29% (95% confidence interval: 27-31%). One percent of the study dairy cattle had the subcutaneous lesions. There were statistical differences for percentage of test positive against bovine leukaemia virus in dairy cattle between provinces ($p<0.001$). The seropositivity in relation to the existence of subcutaneous lesions indicated statistical significance ($p = 0.023$). Knowledge of the provincial difference of seropositivity against bovine leukaemia virus in dairy cattle would be used to determine the resource allocation for preventive measures in the study area. In the preventive measures, serological tests against bovine leukaemia virus for the cattle with subcutaneous lesions, which would be a potential indicator of the infection of bovine leukaemia, should be prioritised.

Key words: AGID, BLV, logistic regression, odds, Santa Cruz, South America

INTRODUCTION

Enzootic bovine leukaemia is a viral disease of adult cattle, caused by Bovine Leukaemia Virus (BLV) and distinguished by neoplasia of lymphocytes and lymph nodes (Kettmann *et al*., 1994; Kahrs, 2001). Infection occurs by iatrogenic transfer of infected lymphocytes and is followed by a chronic antibody response and, less often, development of persistent lymphocytosis or lymphosarcoma (Radostits, 2005). This form is seldom seen in cattle less than two years of age and increases in incidence with increasing age (VanLeeuwen *et al*., 2001). The infection does not spread quickly, but the number of seropositive animals may be 80% in infected herds (Gutiérrez *et al*., 2009). Dairy cattle are more frequently infected than beef cattle and have a higher incidence of lymphosarcoma

Corresponding Author: K. Suzuki, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata, B1900AVW, Argentina
Tel/Fax: +54 221 425 3276

(Radostits, 2005). The annual mortality risk may be as high as 5% in severely affected dairy herds (Acaite *et al*., 2007). All breeds of cattle are susceptible to bovine leukaemia virus infection (VanLeeuwen *et al*., 2001). The prevalence of infection in a cattle population may be high (Jacobs *et al*., 1991; Sargeant *et al*., 2001; Trono *et al*., 2001; Ott *et al*., 2003; Monti *et al*., 2005), but only a few animals develop fatal lymphosarcoma (Radostits, 2005). In Bolivia, field investigations to determine seroprevalence against bovine leukaemia virus were previously implemented which were simply descriptive studies (Ruiz *et al*., 2009). No quantitative epidemiological investigations to identify potential risk factors for the infection have been published to the best of the authors' knowledge. The objective of this study was to (1) determine seroprevalence against bovine leukaemia virus and (2) investigate the risk factors with the seropositivity of the virus in dairy cattle in Bolivia.

MATERIALS AND METHODS

This study was conducted between April and September 2009. Santa Cruz Department (study area) has about 72, 000 dairy cattle, equivalent to 69% of the total dairy cattle population in Bolivia (Federación Departamental de Productores de Leche, 2009, personal communication). One, two, one, two and three dairy cooperatives were selected from Andrés Ibáñez, Warnes, Obispo Santiesteban, Sara and Ichilo Provinces in Santa Cruz Department, respectively. Ten different dairy farms from each of the dairy cooperatives were recruited. In total, 90 farms of dairy cattle were initially planned for visit. Cattle greater than or equal to two years of age were targeted because bovine leukaemia with a predilection for those cattle (Gonzalez *et al*., 2007; Panei *et al*., 2009). The required sample size of 1505 in total from a dairy cattle population of 72, 000 was sufficient to obtain a 95% confidence interval with a desired precision of ±2.5% when the estimated seroprevalence was 50% (Hintze, 2008). The sample size of 20 in each of the farms was assigned by available financial, human and material means. The field study consisted of data collection through questionnaire interviews for each study farm, in combination with blood sample collections and inspection of skin to find a subcutaneous lesions (>1 cm diameter) for each dairy cattle. A questionnaire was designed to obtain basic information of a farm such as the number of dairy cattle reared, age, breed and number of calving of cattle. Blood samples were used for diagnostic tests. Individual-cattle sera were analysed using a kit of agarose gel immunodiffusion for the detection of antibody against bovine leukaemia virus, provided by Cátedra de Virología, FCV-UNLP. Data were entered into a database using the Base in the OpenOffice.org software version 3.1.1 (Sun Microsystems, Santa Clara, CA, USA). The statistical analyses were performed using Stata SE 10.1 (Stata Corporation, College Station, TX, USA). Univariate and multivariate analyses were used to describe the differences between the two dairy cattle groups categorised according to seropositivity against bovine leukaemia virus. Univariate analysis were conducted using Pearson's Chi-squared statistic for categorical predictors such as Province, Breed (Holstein-Friesian vs. others) and Subcutaneous lesions and likelihood-ratio statistic for continuous predictors such as Age and Calving. Following the univariate analyses, a multivariate logistic regression analysis (Eyduran *et al*., 2005) was conducted to better understand the relationships between the outcome Seropositivity and the predictors mentioned above. In the analysis, the most important predictors differentiating between the two dairy cattle groups were identified, based on inclusion of all variables, which were significant at $p<0.10$ in the univariate analysis. A disadvantage of the univariate analysis was that a set of variables, of which each is weakly associated with the outcome, can become important predictors when they are taken together. To prevent this, a significance level that was relatively safe ($p<0.10$) was selected (Noordhuizen *et al*., 2001). The term Subcutaneous

lesions was forced into the model because it was known as a clinical sign of bovine leukaemia (Radostits, 2005). A stepwise backward variable selection approach was used based on the likelihood-ratio statistic and entry and removal probabilities of $p<0.05$ and 0.10, respectively. The model goodness-of-fit was assessed using the Hosmer-Lemeshow goodness-of-fit test. The test P value closer to 1 indicates better fit. All variables included in the final regression model were screened for possible interactions and in case of continuous variables for non-linearity of effects (Katz, 1999).

RESULTS

The 98 study farms (equivalent to 14% of the farms registered in dairy cooperatives of the study provinces) had 12, 404 dairy cattle in total at the visit (equivalent to 17% of the total dairy cattle population in Santa Cruz Department). The number of dairy cattle reared was variable between the study farms (range: 9-630, median: 90). Blood samples collected from 1823 dairy cattle, on the basis of different sample sizes per farm (range: 7-20, median: 20), were serologically investigated. The study dairy cattle had the median age of six years (range: 2-17 years) and the median number of calving of three (range: 0-12). With respect to breed, about 30% of them were Holstein-Friesian (called as Holando), followed by crossbred, Brown Swiss, Criollo and etc. One percent of them had the subcutaneous lesions. Table 1 shows descriptive statistics for percentage of test positive against bovine leukaemia virus in the study dairy cattle between provinces, the statistical precision was improved from ±2.5 to ±2.1% because of the eventual total number of samples of 1823 (larger than planned) and the overall percentage of test positive of 29% (smaller than expected). There were statistical differences for percentage of test positive against bovine leukaemia virus in dairy cattle between provinces ($p<0.001$). The seropositivity in relation to the existence of subcutaneous lesions indicated statistical significance ($p = 0.023$). While, the seropositivity in relation to age, breed or number of calving did not indicate statistical significance. On the basis of the results, predictors Province and Subcutaneous lesions were included in the final multiple logistic regression model (Table 2). The model represented a very good fit to the

Table 1: Percentage of test positive against bovine leukaemia virus in dairy cattle in Santa Cruz Department, Bolivia

Province	No. of farms studied/ No. of farms registered in dairy cooperatives	No. of cattle sampled	% of test positive	95% confidence interval	
				Lower	Upper
Andrés Ibáñez	10/37	191	29	22	35
Warnes	23/224	411	38	34	43
Obispo Santiesteban	10/80	194	54	47	61
Sara	20/68	399	22	18	26
Ichilo	35/304	628	18	15	22
Total	98/713	1823	29	27	31

Chi-squared statistic 122.0, 4 df, $p<0.001$

Table 2: Final multiple logistic regression model describing the probability of a dairy cattle having seropositivity against bovine leukaemia virus in relation to provinces where the cattle was reared and the existence of subcutaneous lesions as specific clinical signs (n = 1823)

Results	Odds ratio	95% confidence interval		p-value
		Lower	Upper	
Ichilo (reference)	1.0			
Andrés Ibáñez	1.6	1.1	2.3	0.010
Warnes	2.5	1.9	3.2	<0.001
Obispo Santiesteban	4.8	3.5	6.6	<0.001
Subcutaneous lesions not found (reference)	1.0			
Subcutaneous lesions found	2.1	1.0	4.7	0.059

Hosmer-Lemeshow goodness-of-fit statistic 0.35, 3 df, p = 0.951

data. There were no interactions among predictors. The final model indicated that Obispo Santiesteban, Warnes and Andrés Ibáñez Provinces (in descending order) were more likely to have seropositive-cattle against bovine leukaemia virus, compared with Ichilo Province. Also cattle having subcutaneous lesions were twice as likely to be seropositive in comparison with those without the lesions.

DISCUSSION

The results presented here constitute the first departmental-wide survey carried out to detect antibodies to bovine leukaemia virus in dairy cattle in Bolivia. In summary, during the period April-September 2009, the observed individual prevalence of the antibodies in this study (29%) was similar to that reported by other authors in countries where bovine leukaemia virus infection is endemic (Jacobs *et al*., 1991; Sargeant *et al*., 2001; Trono *et al*., 2001; Ott *et al*., 2003; Monti *et al*., 2005). Trono *et al*. (2001) and Monti *et al*. (2005) reported the seroprevalence of bovine leukaemia virus in cattle in Argentina were 33 and 70%, respectively. In Canada, Jacobs *et al*. (1991) and Sargeant *et al*. (2001) published the seroprevalence of bovine leukaemia virus in cattle of 36 and 52%, respectively. Ott *et al*. (2003) reported the seroprevalence of bovine leukaemia virus in cattle in the United States was 41%. Knowledge of the provincial difference of seropositivity against bovine leukaemia virus in dairy cattle would be used to determine the resource allocation for preventive measures against bovine leukaemia in Santa Cruz Department. The existence of subcutaneous lesions would also be used as a potential indicator to detect positive-cattle with bovine leukaemia (Radostits, 2005). Serological tests against bovine leukaemia virus for those cattle with subcutaneous lesions should be prioritised. This leads to better resource allocation by policy makers for scientific evidence-based preventive measures (Cockcroft and Holmes, 2003).

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