

## Evaluation of natural transmission of bovine leukaemia virus within dairy herds of Argentina

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### SUMMARY

The purpose of this study was to describe patterns of seroconversion to bovine leukaemia virus and to estimate the main parameters needed for future model building. A longitudinal study was carried out between February 1999 and November 2001 in seven commercial dairy farms in Argentina using 1535 lactating cows. Time-interval parameters were analysed using a parametric survival model with shared frailty, time until infection was analysed using a Bayesian interval-censoring survival model and the infection transmission parameter ( $\beta$ ) was estimated by a generalized linear model. The reproduction ratio ( $R_0$ ) was calculated. In total, 1000 cows tested positive and 494 tested negative. The predicted median age at infection was 4·6 years for seroconverted cows. For infected herds, the proportion of positive calves was as high as for infected cows and showed a large proportion of infected breeding heifers. Peaks in the overall average incidence per season-year were observed during autumn and spring. Results reveal that the period around parturition is a high-risk period. Moreover, heavily infected herds seem to have an increased proportion of young stock infected. The overall  $\beta$  was estimated as 2·9/year (95% CI 1·9–3·7) and combined with a relatively long infectious period it resulted in a high reproductive ratio ( $R_0 = 8·9$ ). Therefore, a high effectiveness of control measures needs to be achieved to eradicate the disease.

### INTRODUCTION

Bovine leukaemia virus (BLV) is a retrovirus that together with human T-lymphotropic virus (HTLV) and simian T-lymphotropic virus (STLV) belongs to the *Deltavirus* genus of the family Retroviridae [1]. BLV causes lymphomas and other disorders in cattle [2]. Due to the tendency of being clustered in geographical areas and herds, the disease was for many years referred to as enzootic bovine

leucosis. BLV has a large economic impact on the livestock sector of many countries around the world.

BLV is transmitted horizontally by infected lymphocytes [3, 4] or it is transmitted vertically [5–7]. Most of the time, infection is iatrogenic and occurs when the animals are treated without adequate hygienic care, e.g. when injected [8], dehorned, [9–11] tattooed, [12] ear-tagged, castrated, bled [13] at teat removal and at rectal examinations [14–17]. Transmission by insect bite has also been reported [18–22], especially with insects of the family Tabanidae [23–25]. Although these different routes could not be considered individually, when gathered in a single equation they represent the horizontal transmission process.

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Table 1. *Main characteristics of herds used during the study*

Herd	No. lactating cows (mean $\pm$ s.d.)	Breed	305-day milk production (litres)
A	105 $\pm$ 9	Hostein	5880 $\pm$ 1556
B	73 $\pm$ 22	Holstein and Holstein $\times$ Jersey	5810 $\pm$ 1491
C	130 $\pm$ 17	Holstein	6020 $\pm$ 1454
D	126 $\pm$ 19	Holstein	6020 $\pm$ 1460
E	95 $\pm$ 14	Holstein	5070 $\pm$ 1400
F	40 $\pm$ 8	Holstein	5670 $\pm$ 1235
G	93 $\pm$ 19	Holstein	4662 $\pm$ 1091

For the development of effective BLV control strategies, quantitative information on transmission of BLV in cattle herds is needed such as length of the infectious period, probability of transmission given exposure, etc. This information is currently not available in literature.

The purpose of this study was to describe patterns of seroconversion to BLV and to estimate the main parameters needed for future model building. This information can then be used in simulation models to evaluate various scenarios, which may result in hypotheses about which aspects are important for a control strategy.

## MATERIAL AND METHODS

### Study population

A longitudinal observational study was carried out between February 1999 and November 2001 in seven commercial dairy farms (denoted A–G) in Argentina. Although these herds were selected based on the owner's willingness to collaborate in the study, they are considered typical dairy farms of the area in terms of herd size (province average 101 cows), milk production (province average 46 50l), breed and management policies [26]. The main characteristics of the herds are summarized in Table 1. Animals graze on rotational paddocks all year round and are milked twice a day. It was known that herd B, D, E and F were endemically infected with BLV. During the study, farmers did not receive information on the infection status of their animals.

### Data collection

Data were obtained from farmer's records and the Milk Control Association, which supplied

information on dates of birth or purchase, breed, calving, dry-off and culling or death. Information on pregnancy status and health-related problems was recorded during regular visits (between 3 and 8 weeks) of the veterinarian responsible for monitoring herd reproduction.

### Collection of samples and diagnostic assays

Foremilk samples (5 ml) from all lactating cows in each herd were collected on average every 2 months during milk control sampling. This milk was used to determine the infection status of animals. All samples were transported on ice in cool boxes to the laboratory, where they were stored at  $-20^{\circ}\text{C}$  until processing. At the end of the study four herds (B, C, F and G) were entirely bled, while in herd A only lactating and dry cows, were bled.

Serum and milk samples were tested using a blocking ELISA 108 [27], with an estimated sensitivity of 98.9% [95% confidence interval (CI) 96.4–99.9] and a specificity of 98.0% (95% CI 94.6–99.5) [28]. Serum samples were considered positive when their percentage of inhibition (PI) was  $>40\%$  of the standard positive, inconclusive when the PI was between 35 and 40% and negative when the PI was  $<35\%$ . The cut-off points used for milk samples were slightly higher,  $>52\%$  being considered positive and between 47 and 52% inconclusive.

### Data processing and estimation of parameters

#### Age structure

In dairy farms, animals usually are kept in groups to optimize and facilitate management. To keep the analysis relatively simple but representative of the general situation, three age groups were considered. Group 1 includes all females from birth to 180 days of life. Group 2 includes all females over 181 days but not yet introduced in the milking herd (2 months before the expected day of calving). Group 3 includes all pregnant heifers within 2 months of calving and all cows that had calved at least once. These groups were considered for prevalence estimation using serological results.

#### Age at first calving and culling

Because some of the parameters to be estimated have time as unit, we assessed the potential relationship of those variables and BLV status, using a parametric

survival model with shared frailty to account for heterogeneity between individuals clustered within herd [29]. Briefly, a parametric frailty survival model introduces an unobserved multiplicative effect  $\omega$  on the hazard. Since the hazard function is non-negative,  $\omega$  must be restricted to non-negative values. The linear regression form is:

$$\ln t_{ij} = \mu + x_{ij}\phi + \sigma W_{ij}, \tag{1}$$

where  $t_{ij}$  is the failure time of the  $i$ th individual ( $i = 1, \dots, n$ ) in herd  $j$  ( $j = 1, \dots, q$ ),  $\mu$  is the intercept parameter,  $\phi$  is the unknown vector of regression coefficients (that we want to estimate) and  $x_{ij}$  is a vector of observed covariates. The random vectors  $W_j = (W_{1j}, W_{2j}, \dots, W_{nj})'$ ,  $j = 1, \dots, q$  are usually assumed to be independent of each other but for multiplicative failure time data, their components (within-herd errors) are generally not. The marginal independence approach estimates  $\phi$  while ignoring the possible correlation among components of  $W_j$ . In contrast, by including frailties, the possible correlations among failure times are explicitly modelled. Specifically, we assume that the hazard function of  $W_{ij}$ , conditional on a random variable  $\omega_i$ , for  $j = 1, \dots, q$  and  $i = 1, \dots, n$  is:

$$h_{ij}(t|\omega_j) = \omega_j h_0(t), \tag{2}$$

where,  $h_0$  is an unknown baseline hazard function independent of the covariates  $x_{ij}$  and  $\omega_j$  are the frailties. Conditional on  $\omega_j$ ,  $W_{ij}$  are all independent. All within-herd failure times share the same frailty term  $\omega_j$ , which models common effects of the members of a herd that are not explained by the available covariates. In addition, the frailty term  $\omega_j$  may also model the heterogeneity of the individuals across herds. We assume that  $\omega_j$ 's are an i.i.d. sample from a gamma distribution.

**Probable time to infection and criteria for determining infected animals**

An individual was considered as infected when it showed two or more positive milk-test results during the follow-up period. In the special case that from a given animal only two test results were available and one was positive it was also considered as infected; when one of the results was doubtful and the other negative, the animal was considered as non-infected.

The date of observed seroconversion is not the time of infection. For cows that seroconverted during the

follow-up period, the most likely time of infection can be deduced from the last observation of a negative serological result and the time of the first positive observation,  $t'_1$  and  $t'_2$  respectively. We used a Bayesian approach based on ref. [30] using the concept of interval-censoring survival analysis. Briefly, let  $T_\alpha$  represent the time until seroconversion for an animal after start of follow-up and assume that  $T_\alpha \in (t'_1, t'_2)$ . Then, if  $\theta$  represents the current time until infection, it follows that

$$\Pr(T_\alpha \in [t'_1, t'_2]|\theta) = \Pr(T_\alpha - \theta \in [t'_1 - \theta, t'_2 - \theta]|\theta). \tag{3}$$

The quantity  $T_\alpha - \theta$  is the time until seroconversion from experimental infections, which was derived from a previous study [31]. Assuming that  $T_\alpha - \theta$  has a distribution with survival function  $S_t$ , then eqn (1) is:

$$\Pr(T_\alpha \in [t'_1, t'_2]|\theta) = S(t'_1 - \theta) - S(t'_2 - \theta) \tag{4}$$

and follows a gamma distribution.

We reflect prior knowledge or uncertainty about  $\theta$  in the form of a density  $D(\theta)$ , where  $D(\theta) \geq 0$  and  $\int_0^\infty D(\theta) d\theta = 1$ . Then the posterior density for  $\theta$ ,  $L < \theta \leq U$ , where  $L$  and  $U$  are the lower and upper bounds of the time until infection given the data, takes the following expression:

$$D(\theta|T_\alpha \in [t_1, t_2]) = D(\theta)\{S(t_1 - \theta) - S(t_2 - \theta)\} / \int_L^U D(\theta)\{S(t_1 - \theta) - S(t_2 - \theta)\} d\theta, \tag{5}$$

We obtain the point estimate of  $\theta$  by finding the value  $\theta'$  or the middle value if many, that maximizes the function and we consider it as the estimate of the most probable time until infection.

**Determination of transmission parameters**

*Infection rate parameter*

We assumed that new infections are generated by a (time-dependent) Poisson process within a SI stochastic model. In this model individuals can either be Susceptible (S) or Infectious (I). New infections are assumed to occur at the rate  $(\beta S_t \cdot I_t)/N_t$ , where  $\beta$  is the infection rate parameter and  $N_t$  the number of animals present at time  $t$  ( $N_t = S_t + I_t$ ). To obtain the value of the infection rate parameter  $\beta$  we also assume the following conditions:

- animals are susceptible during their whole life;
- there is no age-dependent susceptibility;

- an infected individual remains infectious until removal or dead with equal infectiousness during this period;
- the coefficient of transmission is constant over time

Hence, the number of new infections ( $C$ ) in the interval  $(t_1, t_2)$ , follows a Poisson distribution with a mean:

$$E\{c(t_1, t_2)\} = \int_{t_1}^{t_2} \tau(\lambda) d\tau. \quad (6)$$

Given the data obtained from the longitudinal study, the number of new cases per sampling period was known and since  $S$ ,  $N$  and  $I$  are known, the coefficient  $\beta$  can be estimated. We used a generalized linear model (GLM) with a log link, and  $\log(S.I/N)$  as offset variable. Then, we have approximately:

$$\log(E\{C(t_j, t_{j-1})\}) = \log(\beta) + \log(1/2 * (t_j - t_{j-1}) * \{S(t_{j-1})I(t_{j-1})/N(t_{j-1}) + S(t_j)I(t_j)/N(t_j)\} * \Delta t). \quad (7)$$

With eqn (7) transmission parameter  $\beta$  was estimated for each farm. To estimate the 95% confidence interval for  $\log \beta$ , we used the standard error (s.e.) as:

$$\log \beta \pm t_{1-\alpha} * \text{s.e.} = \log \beta \pm 1.96 * \text{s.e.}, \quad (8)$$

where  $t_{1-\alpha}$  is the two-side confidence coefficient assuming a normally distributed variable. Finally, results were shown after exponential transform.

The residual deviance of the model and the plot of the deviance residuals against fitted values were used to evaluate goodness of the fit of the model.

The effect of herd on the age at infection and on the age of seropositive young stock was assessed by the log-rank test for equality of survival distributions (Kaplan–Meier estimation). Because the follow-up period covered a relatively long time, the age at infection might be related to changes in prevalence over time. Therefore, the study was divided in two periods of approximately 1 year each and the effect of year on the age at infection was tested using a log-rank test.

### Reproduction ratio ( $R_0$ )

The basic reproduction ratio ( $R_0$ ) is a key parameter in transmission theory as it defines a threshold condition that determines whether an infectious disease will spread in a susceptible population when the disease is introduced into it. The basic  $R_0$  is defined as the average number of secondary cases produced by one infected individual during the individual's entire infectious period when the pathogen is first

introduced [32]. Because there is no previous estimation of  $R_0$  for BLV, we approximate it using the results from this study. For that reason we estimated the reproductive number by the following formula:

$$R_0 = \beta * 1/\gamma, \quad (9)$$

where  $1/\gamma$  is the average infectious period.

We estimated  $1/\gamma$  as the difference between the median age at infection and the life expectancy for BLV-infected cattle because animals remain infected for the rest of their lives.

## RESULTS

### Descriptive analysis

A total of 7961 milk samples and 1009 blood samples from 1535 animals were taken. From all animals, 1000 of them tested positive and 494 tested negative for milk samples. The remaining animals ( $n=41$ ) showed a pattern that consisted of one positive result followed by 4–12 negative results. In addition, we observed that 15% of the animals that were tested positive showed a pattern that consisted of several positive test results with few intermediate negative results. This type of response has been described for BLV-infected animals that are co-infected with bovine virus diarrhoea virus (BVDV) [33, 34]. In total, 1.7% of the negative results of infected animals were from samples taken during peripartum time (4 weeks previous to 2 weeks after calving).

The median age at infection by herd and by year of study was different by log-rank test for equality of survival distributions ( $\chi^2=90.2$ , 9 D.F.,  $P<0.001$ ). For cows that seroconverted, the predicted median age at infection was 4.6 years. The median age at infection was different between herds as indicated by log-rank test for equality of survival distributions ( $\chi^2=48.1$ , 5 D.F.,  $P<0.001$ ). Also the median age at infection in the first year of the study was different (higher) than in the second year (log-rank test for equality of survival distributions [ $\chi^2=55.7$ , 1 D.F.,  $P<0.001$ ]).

Seroprevalence and median age at infection for the three categories of animals are shown in Table 2. The prevalence in calves of herds B and G was as high as the prevalence of infected cows. Infected herds also showed a large proportion of breeding heifers infected (group 2). For the age of infection there is no clear association with prevalence.

The ELISA test used could not distinguish between passively transferred antibodies and those actively

Table 2. Distribution of serological results in three age groups of four selected farms

Age group	Prevalence of positive sero-reactors (%)				Median age for seropositive animals (yr)			
	Herd B	Herd C	Herd F	Herd G	Herd B	Herd C	Herd F	Herd G
Group 1: 0–180 days	71.4	0.0	n.d.	100.0	n.c.	n.c.	n.d.	n.c.
Group 2: 181 days until introduction in milking herd	57.1	0.0	46.1	40.3	1.7	n.c.	2.5	1.2
Group 3: Adult cows and pregnant heifers	77.2	0.0	99.9	99.5	3.4	n.c.	4.7	5.5

n.d., Not determined; n.c., not calculated.

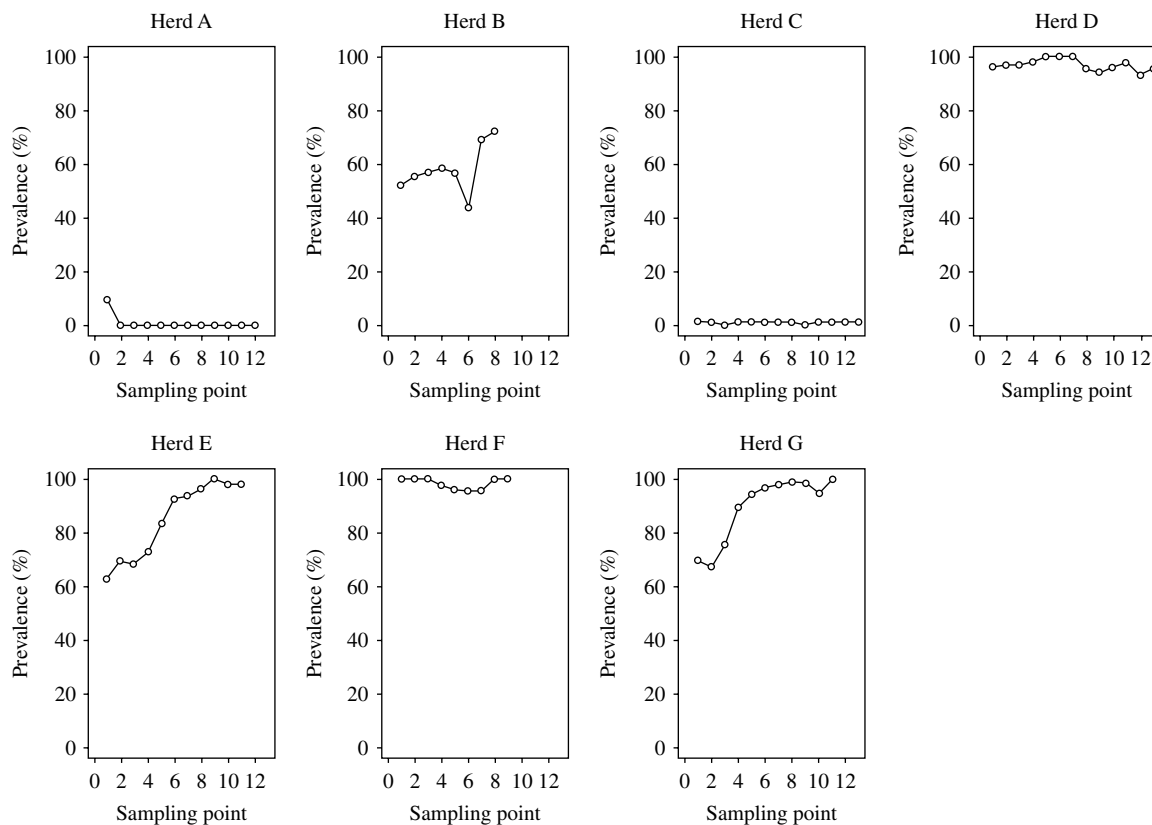


Fig. 1. Seroprevalence by herd during follow-up period (February 1999 to November 2001).

produced after infection. Therefore, seropositive animals of group 1 were excluded from calculations because a positive test result could not always be attributed to infection.

#### Temporal pattern of serological reactivity

The evolution of the prevalence and estimated incidence rates across the follow-up period for each farm is shown in Figures 1 and 2 respectively. The prevalence curves show a steadily increasing trend (herds B, E and G) or small oscillations

at high (herds D and F) or low (herds A and C) levels.

Tendencies of incidence rates could be spliced in two halves for herds B, E, and G representing the first and second year of the follow-up interval. First, the curve reflects a steady increase of the incidence in the first period, followed by high oscillations in the second half. This shift in the shape started when prevalence was > 70%. In highly infected herds (D, F) the oscillations may reflect the very few susceptible individuals. Finally, herds A and C represent a situation of low transmission between individuals.

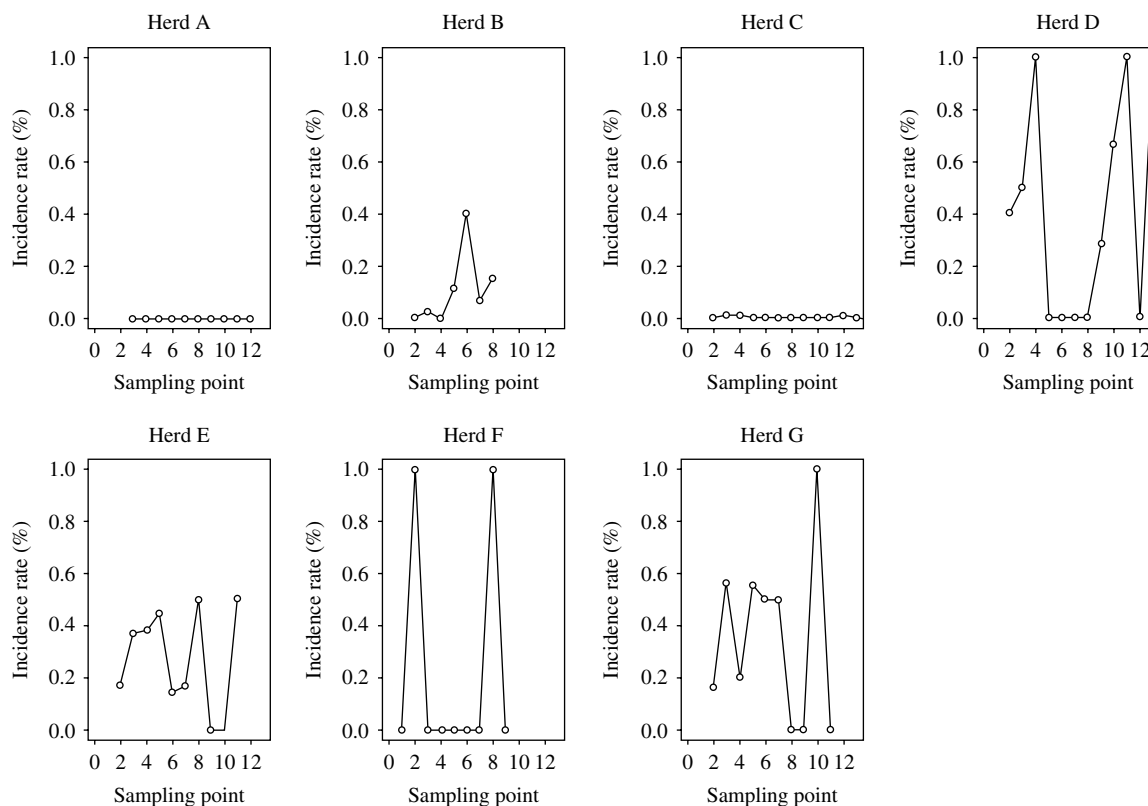


Fig. 2. Incidence rate by herd during follow-up period (February 1999 to November 2001).

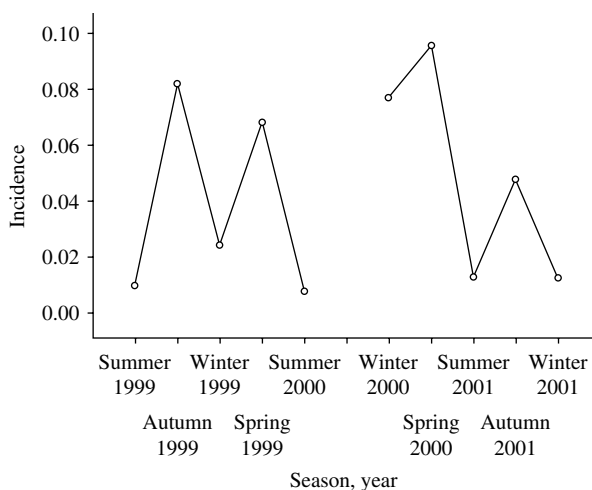


Fig. 3. Seasonal average incidences rates.

Figure 3 shows a plot of the overall average incidence per season-year, peaks during autumn and spring across years were observed.

**Serological status and culling**

Cows that were culled during the follow-up period had a median age of 9.1 years (3322 days). BLV-positive

cows had a median age of 8.3 years (3044 days) and for BLV-negative cows the median age was 9.1 years (3327 days) but the difference was not statistically significant ( $P=0.248$ ).

The median age at first calving was 2.8 years (1010 days). No statistical difference ( $P=0.49$ ) was present between BLV-positive cows (2.8 years/1012 days) and BLV-negative cows (2.7 years/1002 days).

**Transmission parameters**

The date of infection was estimated for each newly infected cow in our dataset and it was used as such for the calculation of the transmission parameter (assuming that BLV-infected animals become infectious in a short time).

The overall estimated  $\beta$  was 2.89/year and the matching 95% CI equalled 1.89–3.74. The estimated  $\beta$ 's and respective 95% CIs by herd are shown in Table 3. When we compare the intervals by herd we see that they extensively overlap, therefore, it can be concluded that although BLV prevalence differed between herds infectiousness did not differ much. Residual deviance of all models and the plot of the

Table 3. *Estimated transmission rates and 95% confidence intervals (CI) for transmission of BLV and the average estimated infectious period of BLV-infected cattle by herd, within six dairy herds of Argentina*

Herd	Transmission rate parameter (year <sup>-1</sup> ) (95% CI)	Average estimated infectious period (yr)	$R_0$
A	n.d.	n.d.	n.d.
B	2.22 (0.22–4.22)	3.96	8.79
C	1.20 (0.0–2.60)	4.24	5.08
D	3.08 (2.41–3.81)	2.88	9.86
E	2.92 (2.34–3.49)	1.95	4.72
F	4.15 (3.50–4.81)	3.81	15.81
G	2.86 (1.99–3.73)	3.38	9.02

n.d., Not determined.

deviance residuals against fitted values (not shown) indicate adequate model fit.

The periods during which cattle were assumed to be infectious are shown in Table 3 and we estimated the reproductive ratios based on their average.  $R_0$  was estimated as 8.88.

## DISCUSSION

The purpose of this study was to describe patterns of seroconversion to BLV and to estimate the main parameters needed for future model building.

Although infected individuals produce a permanent antibody response we found patterns of alternate positive and negative results as reported in previous studies [33, 34]. This may reflect the situation where levels are below the detection level of the test. We did not test for presence of BVDV antigen, but in most of the herds where this pattern was present, farmers recognized the presence of BVDV-infected animals within their herds. We also found patterns of negative results when infected cows were sampled around parturition as previously reported [35–38]. Although this situation has important implications for management decisions from single results of a test, it did not interfere with the aims of our study because several test results from these cows were available.

The figures that represented the changes of seroprevalence and incidences showed that two main types of disease change over time were present in our study. One that characterizes low level of disease or the start of an outbreak, or control measures associated with adequate hygienic care and the other,

representing a more advanced phase of the outbreak starting from moderate to heavily infected herds. Incidence results from either heavily infected herds or from herds that started from a situation of moderate infection force in the second half of the follow-up period may reflect an oscillatory move to an endemic steady-state level of infection. Because we observed the incidence for a relatively short period of time it is not possible to estimate whether this state persists (i.e. quasi-stationary endemic state [39] or conversely if it is a transitory state).

When looking at patterns of new infections over time we observed peaks during autumn and spring, which coincide with major calving patterns as well. Previous studies reported peaks in summer that could be related to an increased activity of haematophagous insects [40] but others failed to find any seasonal trend [41]. As a consequence of our method of herd selection we can not extend our results to the whole population. Therefore, the possible calving-pattern-related peaks need further investigation in the underlying mechanism. The peripartum could represent a high risk for BLV infection due to:

- Depressed BLV immunoglobulin levels in infected cows at parturition [35, 37, 38]. This alteration in the immune system and stress associated with parturition and early lactation may increase the susceptibility of uninfected cows [42]. In addition, infected cows could become more efficient shedders if loss of immune-mediated suppression caused increased viral expression or higher levels of infected cells in the peripheral blood and discharges at parturition.
- More intensive manipulation of cows around calving. Therefore, if proper hygienic measures are not taken, the probability of spread is increased.
- An increased spread of liquids and materials during the moment of calving and some hours after, that might contain infected lymphocytes thus, facilitating transmission.
- Prevailing management systems in Argentina, heifers and cows calving together in the same paddock, and usually sharing the same paddock for at least 2–8 weeks.

Under the conditions of this study the prevalence in heifers was higher than reported before [43] but due to the herd selection procedure and the sample size it can not be extrapolated to a more general situation and this point remains open for further research. The estimated age at infection in young stock seems not to

be related to the prevalence in adult cattle but our estimations were based on a single test moment and, therefore, these results should be taken cautiously. More extensive investigations of the dynamics of the disease in young stock are also needed to clarify potential associations.

Age at infection in adult cattle varied between herds reflecting either different introduction paths, different routes of transmission or different times since introduction. However, we could not relate a definite cause that could explain these differences.

Our estimations of age at first calving are in agreement with other reports from the country [44] but due to the lack of sound information we cannot compare our results of longevity. A report from the Argentine Holstein Breeders Association mentioned that breeding-registered cows have a productive lifetime of five lactating periods.

Although the age at culling was lower in BLV-infected cattle in comparison with non-infected cattle this difference was not significant and it is in accord with previous studies [45, 46].

Characteristics of the disease – life-long infectiousness and relatively low mortality rate – combined with a rather long lifetime of the animals plus the relative young age that animals get infected explains the large estimates of the length of the infectious periods. However, another aspect of crucial importance is to assess whether the infectiousness remains constant over time. From our study we could not obtain evidence for that and it is an aspect for further research. Moreover, only few studies [47, 48] looked into this aspect and using proxy markers of infectivity indicated that although a few days after infection there is an increased infectivity, virus load (defined as amount of virus present in peripheral blood mononuclear cells) remains relatively constant at least until 3 years post-infection.

We do not have any previous reference of the infection rate parameter to compare with but we think that it is a conservative value because data used for calculation was obtained from farms that had an initially moderate to high prevalence when the follow-up started.

We estimated  $R_0$  as 8.8, and no previous estimations for BLV are available for comparison but our estimation is similar to  $R_0$  estimations from another retrovirus (HIV) which ranges from 9 to 12 [49]. Although we chose a very simple model for estimating  $R_0$ , its magnitude shows that a high degree of control is necessary to eradicate BLV.

## CONCLUSIONS

Under the conditions of this study, natural transmission of BLV was observed and results reveal that the period around parturition is an important risk period and that heavily infected herds seem to have an increased proportion of young stock infected. Moreover, we estimated the infection transmission parameter as relatively high and combined with a long infectious period this resulted in a high reproductive ratio. Therefore, a high effectiveness of control measures is required in order to eradicate the disease.

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## DECLARATION OF INTEREST

None.

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