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REGULAR ARTICLES

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

Murat Şevik · Oğuzhan Avcı · Ömer Barış İnce

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Abstract Enzootic bovine leukosis (EBL) which is caused by bovine leukaemia virus (BLV) has an important economic impact on dairy herds due to reduced milk production and restrictions on livestock exports. This study was conducted to determine the BLV infection status in Central Anatolia Region of Turkey, an important milk production centre, and to examine the risk factors such as purchasing cattle, increasing cattle age, cattle breed and herd size associated with transmission of BLV infection. To estimate the rate of BLV infection, a survey for specific antibodies in 28,982 serum samples from animals belonging to 1116 different herds situated in Central Anatolia Region of Turkey were tested from January 2006 to December 2013. A generalized mixed linear model was used to evaluate the risk factors that influenced BLV seroprevalence. Antibodies against BLV were detected in 431 (2.28 %) of 18,822 Holstein and 29 (0.28 %) of 10,160 Brown Swiss cows. Among 1116 herds, 132 herds (11.82 %) had one or more positive animals. Also results of our study show that the prevalence of BLV infection increased from 2006 to 2011, and it tends to reduce with BLV control programme. Furthermore, we found positive associations between percentage of seropositive animal and increasing cattle age, herd size, cattle breed and purchased cattle. Age-specific prevalence showed that BLV prevalence increased with age. These factors should be taken into consideration for control of BLV infection.

Keywords Enzootic bovine leukosis · Dairy cattle · Turkey · Epidemiology · Risk factors · Generalized mixed linear model

M. Şevik (✉)
Molecular Microbiology, Veterinary Control Institute,
42080 Meram, Konya, Turkey
e-mail: dr_muratank@hotmail.com

M. Şevik
e-mail: murat.sevik@gthb.gov.tr

O. Avcı
Department of Virology, Faculty of Veterinary Medicine,
Selçuk University, Konya, Turkey

Ö. B. İnce
Agriculture and Rural Development Support Institution,
Afyonkarahisar, Turkey

Introduction

Enzootic bovine leukosis (EBL) is a disease produced by bovine leukaemia virus (BLV), an oncogenic retrovirus of the family *Retroviridae* and genus *Deltaretrovirus*, closely related to the human T cell leukaemia virus types 1 and 2 (HTLV-1 and HTLV-2) (Kettmann et al. 1994). The disease has a long incubation period and characterized by persistent lymphocytosis, leukaemia, and/or tumours (lymphoma, lymphosarcoma) (Burny et al. 1980; Johnson and Kaneene 1991a).

Infected cattle develop persistent antibody response because infection with BLV is lifelong (OIE 2012). Therefore, the detection of anti-BLV antibodies indicates the presence of the infection source on the farm. Cattle of all ages may be infected but tumours are seen typically in animals over 3 years of age (Van der Maaten and Miller 1990; Kabeya et al. 2001). Economic losses due to BLV infection can come from reduced milk production, decreased reproductive performance, increased replacement costs, veterinary costs and labour requirements (Pelzer 1997).

BLV infection has a worldwide distribution. Some European countries began seriously to emphasize eradication of BLV infection during the 1980s and have reduced the

prevalence of BLV (Johnson and Kaneene 1991b). The first findings about EBL in Turkey were recorded in the early 1960s (Hakioglu 1964). However, it was not considered clinically important in Turkey until the beginning of live animal imports from Europe and South America since BLV serological surveys reveal that the infection is present in North and South America and eastern Europe (Rodríguez et al. 2011; OIE 2012). In Turkey, EBL has been listed as a notifiable disease since 2011, and quarantine and serological diagnosis methods have been used to control disease.

Up to today, many potential routes of BLV transmission have been identified and include both vertical routes, such as in utero infection (van der Maaten et al. 1981), and horizontal routes, such as physical contact (Kono et al. 1983), blood-sucking insects (Ohshima et al. 1981) and blood or other body fluids with blood cell-contaminated devices (Lassauzet et al. 1990). Despite this knowledge, sero-epidemiological status of EBL in dairy cattle in Turkey and its risk factors are poorly understood. This study presents the largest longitudinal study of BLV seropositivity in dairy farms in Turkey and its association with risk factors such as purchasing cattle, increasing cattle age, cattle breed and herd size.

Materials and methods

Study area and sample collection

This research took place in six different provinces (Aksaray, Karaman, Konya, Nevşehir, Niğde and Yozgat) in the Central Anatolia Region. This is a predominantly agricultural region, and the dairy industry is dominated by a large number of family-run small and medium dairy herds. Information about herd size and cattle movements were obtained from the animal registration system (Turkvet System). At each sampled farm, the unique identity of cattle was recorded from their ear tag and linked to the relevant blood sample. The ear tag was matched to the individual cattle data on the Turkvet System to obtain/confirm the following: the date of birth, whether the animal was homebred or purchased, breed and sex. More than 99 % of cattle were matched within the Turkvet System dataset. The main reason that cattle were not matched was because of wrong registry.

Sampled animals were randomly selected from the small (1–10 cattle) and medium-sized family-type farms (11–50 cattle) which voluntarily joined to this survey. Cattle greater than or equal to 1 year of age were targeted because bovine leukaemia with a predilection for those cattle (Johnson and Kaneene 1992; Murakami et al. 2011). Prescapular, axillar, mandibular lymph nodes and skin of sampled cattle were examined by means of tumour formation.

The number of cattle that were sampled on each farm was determined using the following criteria. It was reported that BLV seroprevalence in Turkey was between 0.3 and 11 % (Uysal et al. 1998; Otlu et al. 2001; Tan et al. 2006). Assuming that the lowest seroprevalence of a typical BLV-infected farm was 1 %, the minimum sample size to detect infected herds with 95 % confidence (margin of sampling error was 9 %) was estimated to be five based on sample size calculations described elsewhere (Martin et al. 1987). Therefore, we collected a minimum of five blood samples from small family-type farms ($n=612$) and a maximum of 46 blood samples from medium-sized family-type farms ($n=504$). The age of the cows ranged between 3 and 190 months, with median age of 3.2 years. Thus, a total of 28,982 cattle (18,822 Holstein and 10,160 Brown Swiss) from 1116 dairy farms were subjected to testing. All data were entered into a dataset using R version 3.1.1 (R Development Core Team 2014). All data were screened for errors. When data mismatches were detected, data were rechecked to determine the source of the mismatch and where possible this was corrected.

Blood samples were collected into vacutainer tubes and serum was separated by centrifugation at 2000×g at 4 °C for 10 min. After heat inactivation at 56 °C for 30 min, serum samples were stored at −20 °C until testing.

Serological analysis

Indirect enzyme-linked immunosorbent assay (ELISA) was performed on commercially available microplates for the detection of antibodies to BLV according to the manufacturer's instructions (IDEXX HerdChek Anti-BLV; IDEXX Laboratories, Westbrook, USA), with sensitivity and specificity reported as 98.5 and 99.9 %, respectively (Johnson and Kaneene 1991a). Internal controls were included on each plate to control for batch to batch variation. All samples were run in duplicate. Optical density (OD) values were determined with an ELISA reader (ELx800, Bio-Tek Instruments Inc, Winooski, VT, USA). A sample was defined as positive when the serum-to-positive (S/P) ratio on the ELISA was ≥0.50 (0.5 is the S/P cut-off point recommended by the manufacturer). The BLV ELISA test kit also requires a confirmation of positive tests, using a sample-to-negative host-cell ratio of ≥1.8.

Statistical analyses

In this study, an infected farm was defined as a farm with one or more infected animals. Associations between seroprevalence of BLV and risk factors were initially screened using Mann-Whitney and the Kruskal-Wallis test. Variables with a P value less than 0.05 in these tests were used for multivariate model building. The normality of the distribution of seroprevalence at infected farms was evaluated by the Shapiro-Wilk test. A generalized linear mixed model was used to evaluate the risk factors that influenced seroprevalence of BLV. The

best model was constructed by a stepwise approach, observing the change in Akaike's information criterion (AIC) of each model. The final model was obtained with the minimum AIC and $P<0.05$. The model is described as follows:

$$\text{logit}(p) = \alpha + \beta a + \beta b + \beta p' + \text{RH} + e$$

where p represents the seropositivity value; α is the intercept, β is fixed effects of age (a), herd size (h), purchasing cattle (p'); RH is the random herd effect; and e is the residual variance. All statistical analyses were performed using R version 3.1.1 (R Development Core Team 2014).

Results

Cattle and herd BLV seroprevalence

The data presented in Table 1 show that 1.58 % (95 % confidence interval (CI) 1.4–1.9 %) of cattle were positive among a total of 28,982 cattle tested. The prevalence of BLV infection among animals was 2.28 % (95 % CI 2–2.6 %) in Holstein and 0.28 % (95 % CI 0.2–0.5 %) in Brown Swiss dairy cattle. This difference between Holstein and Brown Swiss cattle was statistically important ($P<0.05$).

Age-specific prevalence showed that BLV prevalence increased with age (Fig. 1). Overall, 94.3 % (434/460) of positive animals were cattle over 2 years old. The prevalence of BLV in adult cattle (>2 years old, $n=18,576$) on dairy farms was 2.34 % (95 % CI 2.05–2.66 %) and significantly higher than that in cattle less than 2 years old ($n=10,406$) (0.25 %, 95 % CI 0.14–0.43 %) ($P<0.0001$). It was determined that most seropositive animals were in the age interval 3–7 years.

In the present study, an infected farm was defined as a farm with one or more infected animals. Based on this definition, 132 herds (11.83 %, 95 % CI 10.06–13.86 %) were found to be infected (Table 2). BLV prevalence was significantly higher in herds with more than 10 cattle ($P<0.05$). In seropositive herds, prevalence of seropositive cattle ranged between 3.1 % (0.08–16.22) and 26.6 % (12.28–45.89).

Table 1 Seroprevalence of BLV among Holstein and Brown Swiss cattle

Cattle	Total	Positive	Prevalence (%)	95 % confidence interval (CI)
Holstein	18,822	431	2.28	2–2.6
Brown Swiss	10,160	29	0.28	0.2–0.5
Total	28,982	460	1.58	1.35–1.85

Generalized linear mixed model results

The final model was obtained with four variables with the smallest value of the AIC (Table 3). Cattle over 2 years old, Holstein breed, medium-sized family-type farms and purchased cattle were considered as risk factors that facilitated the increases in seroprevalence ($P=0.02$, 0.03, <0.001, <0.001). On the other hand, cattle less than 2 years old, Brown Swiss breed, small family-type farms and unpurchased cattle were considered to be protective factors against BLV.

Seroprevalence of BLV infection in Central Anatolia Region of Turkey

Lowest percentage of antibody response against BLV was detected in Karaman with 1 %. Aksaray, Konya, Nevşehir and Yozgat had prevalence values of 1.3, 2.2, 1.4 and 1.4 %, respectively. BLV prevalence among Konya and other provinces was significantly different ($P<0.05$).

The prevalence of BLV in Central Anatolia Region was found to be 1.1 % (29/2457), 1.1 % (42/3714), 1.5 % (46/3116), 1.6 % (60/3654), 1.9 % (79/4255), 2.5 % (77/3116), 2.2 % (86/3894) and 0.8 % (41/4776) in 2006, 2007, 2008, 2009, 2010, 2011, 2012 and 2013, respectively. BLV appears to be spreading particularly among the dairy cattle population during the 2006–2011 and later it tends to reduce.

Discussion

This is the largest sero-epidemiological study of BLV to assess risk factors related to the seroprevalence of BLV on dairy farms in Turkey. Based on our generalized linear mixed model, the significant risk factors were found to be cattle age, cattle breed, herd size and purchased cattle.

Overall, 11.8 % of dairy farms were found to have cattle infected with BLV, and in seropositive herds, prevalence of seropositive cattle ranged between 3.1 and 26.6 %. From 2006 to 2013, prevalence of BLV in Central Anatolia Region was found ranging from 0.8 to 2.5 %. There was a significant difference in the proportion of seroprevalence between the provincial distributions. BLV prevalence among Konya and other provinces was significantly different ($P<0.05$). This result may be explained by the fact that amount of sample size. In this study, we collected sera samples from 8588, 6014, 5579, 4467 and 4334 cattle in Konya, Nevşehir, Karaman, Aksaray and Yozgat, respectively. There were no different management practices applied in these provinces, so it was expected that there would not be a significant impact of environmental risk factors.

The results of this study are consistent with those reported previously. Previous studies on seroprevalence of BLV in different localities in Turkey have reported prevalence rates

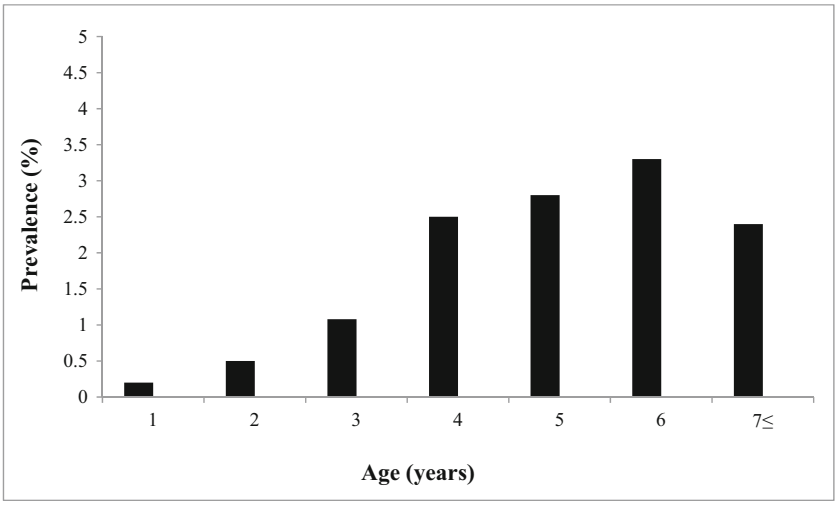

Fig. 1 The distribution of BLV seroprevalence according to age

range from 0.3 to 11 % (Sen et al. 1995; Yilmaz et al. 1997; Uysal et al. 1998; Otlu et al. 2001; Tan et al. 2006). A previous study which was carried out in Turkey reported that herd seroprevalence rates were between 48.3 and 64.7 % (Burgu et al. 1990). A possible explanation for these results may be due to differences in the number of sampled cattle, location of study area and management practices.

BLV has a worldwide distribution and prevalence varies between countries (Nuotio et al. 2003; Acaite et al. 2007). Trono et al. (2001) and Monti et al. (2005) reported that the seroprevalence of BLV in cattle in Argentina were 33 and 70 %, respectively. In Canada, Jacobs et al. (1991) and Sargeant et al. (1997) published that the seroprevalence of BLV in cattle were 36 and 52 %, respectively. Ott et al. (2003) reported that the seroprevalence of BLV in cattle in USA was 41 %. Kobayashi et al. (2010) published that the seroprevalence of BLV in cattle in Japan was 68.5 %. The prevalence rates that were determined in this study are considerably lower than those reported in different regions of the world. Our result is consistent with other reports which indicate that the prevalence of BLV infection in Middle East countries is lower than that in other regions of the world (Hafez et al. 1990; Meas et al. 2000; Pourjafar et al. 2004; Trainin and Brenner 2005; Tan et al. 2006). A possible explanation for the lower prevalence detected in Turkey is the effect of herd size. The herd size is relatively very small in Turkish dairy herds when compared with the European Union (EU) and North and South America. Average size of the dairy farms in Turkey is 5 cows per farm whereas it is 29.4 cows in EU, 62 in Canada and 160 in Argentina (Fuchs 2006; Kaya and Akman 2006; Painter 2007; FAO 2011). Prevalence tends to increase on dairies with increasing herd size. This is because the number of animals that are susceptible to infection is high in large herds.

In Turkey, a nationwide control programme, based on surveillance, control of animal movements and quarantine, against BLV has been established and performed since 2011. We determined that the prevalence of BLV infection ranges from 1.1 to 2.5 % from 2006 to 2011. The increase of prevalence might depend on sanitation conditions and dairy farming practices. Furthermore, dairy cattle were not serologically tested for the disease. Thus, infected and uninfected animals were not separated. Consequently, the virus spreads mainly through the movements of infected animals from one herd to another and within a herd. After the onset of the control programme,

Table 2 Herd prevalence of BLV infection

	No. of herds		Total	95 % confidence interval (CI)
	≤10[a]	>10		
Positive	24 (4 %)	108 (21.4 %)	132 (11.8 %)	9.9–13.8
Negative	588 (96 %)	396 (78.6 %)	984 (88.2 %)	86.1–90
Total	612	504	1116	

[a] Number of cattle

Table 3 Final model for logit-transformed seroprevalence of BLV

Variable	Category level	β[a]	SE[b]	z value	P of z value
Intercept		−2.66	1.83	−0.014	<0.001
Age (years)	<2	Ref.[c]			
	≥2	0.73	0.32	2.28	0.02
Breeds	Brown Swiss	Ref.			
	Holstein	1.54	0.21	7.14	0.03
Herd size	≤10	Ref.			
	>10	1.23	0.15	8.14	<0.001
Homebred		Ref.			
Purchased		1.91	0.15	0.04	<0.001

[a] Estimated coefficients

[b] Standard error for the coefficient

[c] Reference category

prevalence of BLV in Central Anatolia Region tended to reduce (2.5, 2.2 and 0.8 % in 2011, 2012 and 2013, respectively).

Control programmes for BLV exist in several countries, and BLV infection is commonly diagnosed by the detection of antibodies in serum or milk samples. The serological methods currently used for the detection of infected animals are the agar gel immunodiffusion (AGID) test and the ELISA. The ELISAs have much higher sensitivity than the AGID and are more suitable for large-scale testing (OIE 2012). Therefore, in this study, we used ELISA for the detection of infected animals.

With regard to age factor, over 2 years old was found to be positively associated with the seroprevalence of BLV compared with less than 2 years old (P=0.02). A possible explanation may be that longer lifespan increases the probability of BLV exposure, which leads to a higher prevalence of BLV infection. Similarly, previous studies have reported a positive association between antibody prevalence and age (Johnson and Kaneene 1992; Murakami et al. 2011), but in contrast with those observed, Uysal et al. (1998) have found no relationship between age and BLV infection. The lack of an association between seropositivity and age in their study may be a result of the low number of animals left after excluding those from noninfected herds.

Our results showed that the BLV prevalence was significantly higher in Holstein breed in comparison to Brown Swiss breed (P=0.03). Most of the cattle in a dairy herd in Central Anatolia Region are Holstein. A good food conversion ratio (ability to turn feed into milk), the highest average milk production and the greatest content of protein in milk make this breed a very popular choice for dairy farmers (Wendorff and Paulus 2011). Therefore, the numbers of Holstein cattle are more than those of Brown Swiss cattle in dairy herds and they usually live longer than Brown Swiss cattle, so the probability of BLV exposure in Holstein cattle is much than that in Brown Swiss cattle.

In Central Anatolia Region, most of the farms are small- and medium-sized family farms. Dairy production in Central Anatolia Region is based on a loose housing system which causes an increased physical contact with each other. The results of this study showed that the BLV prevalence was significantly higher in herds with more than 10 cattle (P<0.001). Loose housing is a risk factor for transmission of BLV (Kobayashi et al. 2010). Infected cattle can easily contact with uninfected cattle in loose housing system because cattle randomly move in cattle shed and field. Therefore, potential risk of direct and indirect contact between infected and uninfected cattle in larger herds increases the BLV prevalence.

Analyses of risk factors for the seroprevalence of BLV revealed that the presence of purchased cattle from other farms was a risk factor. An uncontrolled cattle movement is the most important risk factor for transmission of a disease from one farm to another. In Turkey, it is thought that farm owners rarely test animals for BLV infection before introducing cattle to the herd. In this study, we determined that approximately 60 % (79/132) of infected dairy farms had introduced cattle from other farms. To prevent the introduction of infected cattle onto a farm, control measures such as negative confirmation of BLV should be done by diagnostic tests.

Conclusions

Control strategies for BLV infection at dairy farms should focus on these risk factors. Quarantine measures should be applied to purchase cattle. Uninfected cattle should be prevented from coming into contact with cattle originating from herds that are not certified BLV free or cattle with unknown BLV infection status.

Conflict of interest The authors declare that they have no conflict of interest.

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