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Prevalence of Bovine Leukemia Virus (BLV) Antibodies in Bulk Tank Milk of Dairy Cattle Herds of Mashhad Area, North-East of Iran

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Abstract: Bovine Leukemia Virus (BLV), a member of retroviridae is an oncovirus that causes a chronic infection in cattle called Enzootic Bovine Leukosis (EBL) and has a worldwide distribution but its overall prevalence in Iran is unknown. EBL causes significant economic loss associated with the cost of control and eradication, loss in milk production and difficulties in exports. The bulk tank samples were collected from 92 dairy herds in Mashhad area during Summer, 2009. The dairy herds were categorized based on the type of herd, herd size and geographical location of herd. A positive ELISA antibody response was detected in 38 (41.3%) out of 92 herds. There was a significant and positive correlation between herd size and PP value (r_s = 0.345, $p<0.01$). This showed that larger herds had higher antibody levels in bulk tank milk and probably higher within-herd prevalence. BLV prevalence was significantly higher in herds with >100 cattle ($p<0.01$). Also, there was a significantly higher BLV prevalence in industrial dairy herds compared with semi-industrial dairy herds ($p<0.05$). BLV prevalence between dairy herds of two regions was not significantly different ($p>0.05$). This study revealed that BLV infection in dairy herds of Mashhad area is influenced by herd size and type of herd. The test showed that it can be used in an extensive investigation for rapid screening of dairy herds in Iran. The researchers predict a major role for herd management practices in this prevalence but characterization of key risk factors needs more investigations.

Key words: Bovine leukemia virus, bulk tank milk, dairy cattle, indirect ELISA, prevalence, Iran

INTRODUCTION

Enzootic Bovine Leukosis (EBL) is an infectious lymphoproliferative disease of cattle caused by Bovine Leukemia Virus (BLV), an exogenous C-type oncovirus which is classified into the genus Deltaretrovirus in the family Retroviridae (Murphy *et al*., 1995). The disease develops in three pathological forms: Asymptomatic course, Persistent Lymphocytosis (PL) and lymphosarcoma (Trono *et al*., 2001). The majority of infected animals remain clinically asymptomatic throughout their life with no apparent negative economic effects but approximately 29% of BLV carriers develop persistent lymphocytosis and <5% of BLV-infected cattle develop lymphosarcoma (Ferrer, 1979). The virus does not circulate in blood but provirus becomes integrated into the genome of milk and blood lymphocytes and tumor cells and are found in the cellular fraction of various body fluids which allows transmission via milk, congenital transmission and most commonly, iatrogenic, horizontal transmission of the disease (OIE, 2008). BLV infection has a worldwide distribution (Schwartz and Levy, 1994) and is listed by the World Organization for animal health (OIE) as a disease of importance to international trade (OIE). The disease is included in the national eradication program in Australia and some member states of the European Union (EU), several of which have eradicated the disease (Nuotio *et al*., 2003; Acaite *et al*., 2007). Because there is no vaccine to prevent infection or progression of the disease, virus specific antibodies found in milk or serum are a good indicator of infection and a practical method for disease screening (Evermann, 1992; OIE, 2008). These antibodies directed towards the envelop glycoprotein gp 51 and viral capsid protein p 24 are found in milk and blood about 3 weeks after infection (OIE, 2008). Enzyme Linked Immunosorbent Assay (ELISA) and Agar Gel Immunodiffusion (AGID) assays approved by OIE for trading purposes (OIE, 2008). ELISA

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has a better turnaround time and higher sensitivity and it has been used as screening test to identify infected dairy herds in regional EBL eradication programs (Eloit *et al*., 1990; Hayes and Burton, 1998; Nuotio *et al*., 2003). The objective of this study was to estimate the prevalence of BLV infection in dairy herds in Mashhad area of Iran.

MATERIALS AND METHODS

Study area, population and sampling: Mashhad is located in Khorasan Razavi province in North-East of Iran and is the second centre of dairy production in Iran. According to the previous study by Musavi, the prevalence of BLV infection in dairy cattle herds of Mashhad area was 39.22%. Therefore, the sample size required for the study with the level of confidence of 90%, desired absolute precision of 10% and an expected prevalence of 39.22%, the minimum required sample size was 92 dairy herds using the following formula (Thrusfield, 2005):

$$n = 1.96^2 P_{exp} (1 - P_{exp}) \div d^2$$

Where:

n = Required sample size
P_{exp} = Expected prevalence
d = Desired absolute precision

The bulk tank samples were collected from 92 dairy herds at two regions in Mashhad area at Summer, 2009. The herds were selected according to a proportional geographical distribution in various parts of Mashhad area. The total number of lactating cows in these herds was 3404 and the study population was about 12.31% of total dairy cows in Mashhad area. The dairy herds were categorized based on the type of herd (Industrial, semi-industrial), herd size (≤100, >100) and geographical location of herd (West, East). Bulk tank samples were collected in 50 mL screw-top plastic tubes after complete mixing in bulk tank. Samples were combined in approximate proportions from different tanks of one herd and no preservative was used. The samples were put on ice and transported to the laboratory. The presence of fat may affect the Optical Density (OD) measurement used with ELISA, so milk samples were first centrifuged at 4000 rcf for 15 min at 4°C in the laboratory and then, skimmed milk stored at -20°C before analysis by the Svanova® BLV gp 51-Ab ELISA kit (Svanova Biotech, Uppsala, Sweden).

Enzyme-Linked Immunosorbent Assay (ELISA): The Svanova® BLV gp 51-Ab ELISA is an indirect ELISA in which microtitre plates are coated with BLV antigen and can be used for bulk milk samples. They were used for detection of antibodies against BLV in bulk milk samples according to the procedure of the manufacturer and validated protocol. The Sensitivity (Se) and Specificity (Sp) of the ELISA test were 100 and 99.4%, respectively. It was not possible for us to determine Se and Sp under the tested bulk milk of the dairy cattle herds in Mashhad area and so the researchers used the values mentioned in the kit. Positive, negative and blank controls and the samples were run in parallel. Optical Density (OD) values were determined at 450 nm with an EL×800 absorbance microplate reader (BioTek Instruments, USA). Before interpretation of the results, all OD values in wells coated with BLV gp 51 viral antigen were corrected by subtracting the ODs of negative control from the samples Ods (Od_{sample} - $Od_{control}$ = $Od_{corrected}$). Percent Positivity values (PP values) were evaluated. All corrected OD values for the test samples and the negative control are related to the corrected OD values of the positive control as follows:

$$PP = \frac{\text{Test sample or negative control } (OD_{corrected})}{\text{Positive control } (OD_{corrected})} \times 100$$

PP value equivalent or >5 were considered positive for BLV infection.

Statistical analysis: Statistical analyses were performed using the SPSS package Ver. 12 (Chicago, Il, USA). Herd size and PP value relationship was detected by Spearman's rho. The Chi-square (χ^2) test was used to evaluate the effect of herd size category, type of herd and geographical location of herd between positive and negative herds.

RESULTS

Total number of cattle and lactating cows in this study that was performed on 92 dairy herds was 7975 and 3437 cows, respectively (Table 1). A positive ELISA antibody response was detected in 38 (41.3%) out of 92 herds.

There was a significant and positive correlation between the herd size and PP value (r_s = 0.345, $p<0.01$) (Fig. 1). This showed that larger herds had higher antibody levels in bulk tank milk and probably higher within-herd prevalence.

BLV prevalence was significantly higher in herds with >100 cattle ($p<0.01$) (Table 2). Also, there was a

Table 1: Descriptive statistics of dairy herds and PP value

Categories	N	Range	Min.	Max.	Sum	Mean±SD
Herd size	92	943	7.00	950	7975.0	86.7±158.5
Lactating	92	368	2.00	370	3437.0	37.4±61.40
PP values	92	93	0.01	93	2356.7	25.6±32.50

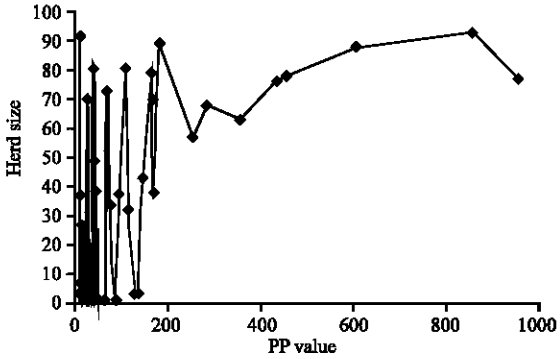

Fig. 1: Correlation between PP value and herd size

Table 2: BLV prevalence in two herd size categories

	Herd		
Results	≤100	>100	Total
Positive (%)	24 (32.9)	14 (73.7)	38
Negative (%)	49 (67.1)	5 (26.30)	54
Total	73.0	19.0	-

Table 3: BLV prevalence in two herd types

	Herd		
Results	Industrial	Semi-industrial	Total
Positive (%)	22 (55)	16 (30.8)	38
Negative (%)	18 (45)	36 (69.2)	54
Total	40	52.0	-

Table 4: BLV prevalence in two regions

	Herd		
Results	West	East	Total
Positive (%)	10 (43.5)	28 (40.6)	38
Negative (%)	13 (56.5)	41 (59.4)	54
Total	23.0	69.0	-

significantly higher BLV prevalence in industrial dairy herds as compared with semi-industrial dairy herds ($p<0.05$) (Table 3). BLV prevalence between dairy herds of two regions was not significantly different ($p>0.05$) (Table 4).

DISCUSSION

Mashhad is a mountainous city and is one of the major livestock husbandry centers in Iran. Previous studies showed prevalence of BLV infection in several cities in Iran (Sarmast 1996; Phirouzi and Bakhshesh, 1999; Momtaz and Hemmatzadeh, 2003; Hemmatzadeh, 2007; Tolouei *et al*., 2009) but this is the first survey that is performed on bulk tank milk by using ELISA test. This study that was performed on 92 dairy herds in Mashhad area showed a BLV prevalence of 41.3%. This is consistent with the results of a serological survey in Mashhad in 2007 which showed a BLV prevalence of 39.22% in dairy herds.

In the present study, there was a significant and positive correlation between herd size and BLV antibody concentration in bulk tank milk. In addition, the prevalence of BLV in dairy herds with less and >100 cattle per herds were 32.8 and 73.6% respectively. Intensive dairy production in large dairy herds in Mashhad area which is based on a loose housing system caused an increased physical contact among cattle. On the other hand, it was reported that BLV transmission occurs primarily through physical contact (Kono *et al*., 1983; Lassauzet *et al*., 1991). In a recent study, Kobayashi *et al*. (2010) showed that a loose housing system is a BLV transmission risk factor. Therefore, positive correlation of BLV infection with herd size could be an effect of higher density in herds with >100 cattle.

Statistical analysis showed that BLV prevalence in industrial dairy herds was higher than that for semi-industrial dairy herds. Previous study by Musavi showed higher prevalence of BLV infection among Holstein cattle in Mashhad area, so it was expected a higher prevalence of infection in industrial dairy herds in this survey. This can show that Holstein cattle are predisposed more than cross breed (Holstein cattle crossed with native cattle) to BLV infection.

There was no significant difference in prevalence between East and West of Mashhad. There is no different management practice between these regions, so it was expected that there would not be a significant impact of environmental risk factors.

This study showed high prevalence of BLV infection in dairy herds in Mashhad area. There are several risk factors that are related to different management practices of dairy herds in Mashhad area that should be considered for the prevention and control of BLV infection. With regard to housing condition, a loose housing system was found to be positively associated with seroprevalence compared with a tied housing system (Kono *et al*., 1983; Lassauzet *et al*., 1991). Loose housing system can increase the chances of contact between uninfected and infected cattle.

Dehorning is a practice in daily herd management in dairy herds in Mashhad area. Studies showed that calves dehorned with contaminated dehorning apparatus had an increased risk of infection than those that had not been dehorned (DiGiacomo *et al*., 1985, 1987; Lassauzet *et al*., 1990).

Another possible method of BLV transmission within-herds can be through hematophagous insects (Bech-Nielsen *et al*., 1978; Ohshima *et al*., 1981; Manet *et al*., 1989). There is no information about horsefly populations and its impact on BLV infection in dairy herds

in Mashhad area but it can be an important risk factor associated with the prevalence of infection.

Studies showed that milk and colostrum from infected cattle can contain BLV infected cells (Ferrer *et al*., 1981; Chung *et al*., 1986). However, all BLV positive cattle do not produce infected milk at all times (Straub, 1982). Since, colostrum contains BLV antibodies, ingestion of colostrum from infected cows reduced the risk of infection during the weaning period in calves (Van Der Maaten *et al*., 1981; Lassauzet *et al*., 1989; Nagy *et al*., 2007). However, it has been reported that feeding of infected bulk milk can cause infection in neonatal calves, especially from healthy dams (Miller and Van Der Maaten, 1979). It was reported that BLV transmission rates in calves by 6-12 months of age can be related to milk born infection at approximately 6-16% in dairy herds (Hopkins and Digiacomo, 1997). In Mashhad, after calves get colostrum from their dams, they are fed by pooled milk which can transmit the BLV from infected cattle to calves. This common practice of feeding bulk milk to calves, unprotected by maternal antibody is likely to be a major factor for the transmission of BLV infection in dairy herds (Dimmock *et al*., 1991). Colostrum feeding could be an effective way for reducing the infection in dairy herds.

Dairy herds should provide optimal conditions for the treatment of pooled colostrum such as heating and freezing on the herd and bulk milk from uninfected cows should be fed to calves.

CONCLUSION

In this study, the researchers showed for the first time in this study that a large number of dairy herds in Mashhad area are infected with BLV. In addition, prevalence of infection has increased as the intensive production system is followed to heighten milk production through increasing herd size. Therefore, the focus of BLV transmission prevention and control programs should be on the herd management practices. Furthermore, in order to prevent BLV transmission, feeding pooled colostrum and bulk milk without the treatment to calves is not recommended. There is room for more investigations to elucidate the key risk factors of BLV transmission in an attempt to control new infections in dairy herds in Mashhad area.

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