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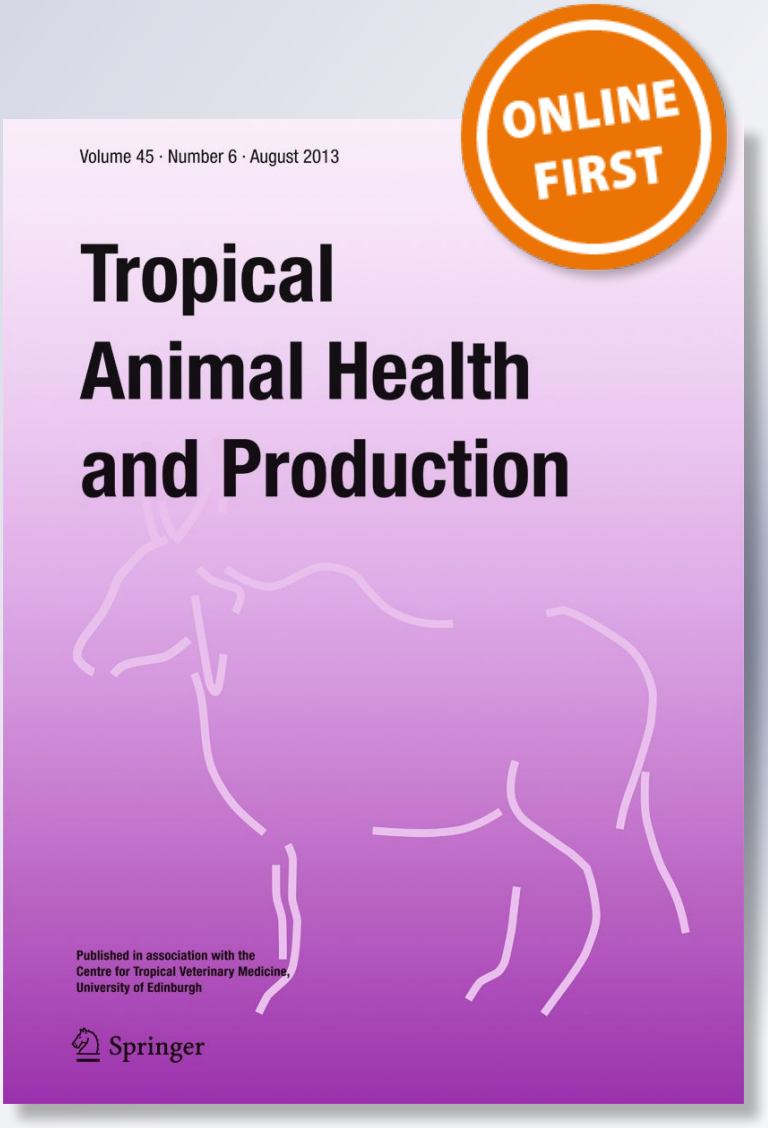

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REGULAR ARTICLES

Seroprevalence of bovine leukemia virus in cattle, buffalo, and camel in Egypt

Abdelfattah Selim[1] · Marawan A. Marawan[1] · Abdel-Fattah Ali[2] · Eman Manaa[3] · Hassab Allah AbouelGhaut[4]

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Abstract

Bovine leukemia virus (BLV) is the causative agent of enzootic bovine leukosis. It causes significant economic losses associated with losses due to slaughter and eradication of infected animal from infected area and other indirect economic losses such as restriction on importation of animals and semen from infected area. The main objective of this study was to determine the seroprevalence of BLV antibodies in cattle, buffaloes, and camels in Egypt using ELISA test. Serum samples were collected from 350 cattle, 100 buffaloes, and 100 camels during 2018. The seropositivity for BLV-specific antibody was 20.8%, 9%, and 0% in cattle, buffaloes, and camels, respectively. The result revealed significant association ($p < 0.05$) between age and seroprevalence of BLV infection in cattle > 4 years (24%) compared with those < 4 years (13%). We found no significant association between pregnancy and herd size and seroprevalence of BLV infection in this study ($p > 0.05$). Furthermore, the age, pregnancy state, and herd size had significant effect on seroprevalence of BLV infection in buffaloes. This study contributes that BLV is detected in cattle and buffaloes in Egypt and confirms that the camels has resistance against BLV infection. Hence, the control measures are very necessary to combat the transmission of the disease and reduce its economic impact.

Keywords BLV · Cattle · Buffalo · Camel · Seroprevalence

Introduction

Bovine leukemia virus (BLV) is considered to be one of the important viruses of bovine lymphotropic virus infection, and is the causative agent of enzootic bovine leukosis (Rovnak et al. 1991). The disease is not only mostly affecting dairy cattle but also frequently seen in buffaloes and camels (Meas et al. 2000; Saidi et al. 2018).

BLV causes economic losses for cattle industry directly, such as decrease in milk production and decrease cow longevity premature culling, and causes economic losses indirectly such as importation restriction of animals from BLV-infected localities (Nishimori et al. 2016).

The most important sources for infection by BLV are blood lymphocytes and other tissue products of infected cattle (Mekata et al. 2015). The disease is not only transmitted mainly by horizontal routes but also may be transmitted vertically by ingestion of colostrum or in utero infection (Esteban et al. 2009). Most BLV-infected cattle (about 70%) do not show any clinical symptoms, and about 30 % of them develop persistent lymphocytosis (PL) and 1–5 % of the infected cattle develop malignant B-cell lymphosarcoma causing EBL (Pandey et al. 2017).

BLV infection is a worldwide-distributed disease; it is highly prevalent in North and South America, Asia, and Eastern Europe. The prevalence of BLV in the Middle East was lower in comparison with other regions in the world except in Turkey and Iran with 48.3% and 64.7%, respectively (Rodríguez et al. 2011).

In Egypt, BLV was detected in imported dairy cattle in 1989 in Arab El-Aoumar, Assiut, with seroprevalence rate of 37.7% in cattle under 2 years old and 72.8% in animals more than 2 years old (Zaghawa et al. 2002), In addition, its

✉ Abdelfattah Selim
Abdelfattah.selim@fvtm.bu.edu.eg

[1] Department of Animal Medicine (Infectious Diseases), Faculty of Veterinary Medicine, Benha University, Banha, Egypt

[2] Department of Clinical Pathology, Faculty of Veterinary Medicine, Benha University, Banha, Egypt

[3] Animal and Poultry Production, Department of Animal Wealth Development, Faculty of Veterinary Medicine, Benha University, Banha, Egypt

[4] Animal production, Research institute, Agriculture Research Center, Ministry of Agriculture, Dokki, Giza, Egypt

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prevalence rate in Kafr El Sheikh, Alexandria, and Menofia governorates was 15.83% (Zaher and Ahmed 2014).

The screening of the disease by detection of specific BLV antibodies in serum and milk is a good indicator of the disease due to lack or absence of vaccination (Ali et al. 2019; Selim and Gaede 2015). Cattle infected with BLV will produce antibodies against the major internal (p24) and envelope (gp51) virion proteins in their serum and milk; hence, antibody-based tests are commonly used for the diagnosis of BLV infection in cattle over 6 months of age (Constable et al. 2016).

Enzyme-linked immunosorbent assay (ELISA) is a rapid method and used as a screening test for BLV because it has sensitivity varied from 97 to 100% and specificity from 78 to 100% (Mousavi et al. 2014; Elhaig et al. 2017). Therefore, the present study was aimed to determine the seroprevalence of BLV antibodies in cattle, buffaloes, and camels in Egypt based on the commercialized IDEXX Leukosis Serum Screening Ab Test (IDEXX laboratories, Maine, USA).

Material and methods

Animals and samples

Animals were selected by using a random sampling strategy at two levels. Sample size at the farm level was fixed so as to estimate an expected herd prevalence of 50% at the 95% confidence level with an allowable error estimated at 10%. In the selected herds, a number of individuals sufficient to detect an intra-herd prevalence of 15–20% were chosen randomly for blood collection.

The screening of antibodies against BLV infection in different animal species (cattle, buffaloes, camels) was performed during 2018. A total number of 350,100 and 100 serum samples were collected from cattle, buffaloes, and camels. The samples were collected from three governorates (Kafr El Sheikh, Qalyubia, and Menofia) depending on the population density of animal species in each governorate.

The examined animals were from both sexes with ages ranged between < 3 and > 4 years and from pregnant and non-pregnant animals. The prevalence BLV infection was studied among animals reared in herd size < 200 and > 200.

Blood sample (5 ml) was collected from jugular vein of each animal using a vacuum tube. The serum was separated from clotted blood by centrifugation at 10,000× *g* for 10 min. All sera were stored at − 20 until tested.

Enzyme-linked immunosorbent assay

All serum samples were examined serologically using the IDEXX Leukosis Serum Screening Ab Test (IDEXX laboratories, Westbrook, Maine, USA) to detect antibodies against BLV according to manufacturer's instructions.

Results were expressed as samples to positive percentage (S/P %), as recommended by the manufacturers. Samples with an S/P % greater than or equal to 60% were classified as positive for BLV antibodies. The obtained data was analyzed to determine the relation between age, pregnancy, herd size, and seropositivity.

Statistical analysis

The chi-squared test was performed to determine the difference between categories. The values of $p < 0.05$ were considered significant.

Results

The seroprevalence of BLV between animal species was significantly different ($p = 0.0001$) in cattle, buffaloes, and camel as summarized in Table 1. The antibodies against BLV infection were not detected in all camels under the study. Moreover, the BLV infection in cattle and buffaloes was more prevalent in Kafr El Sheikh Governorate in comparison with other localities.

In addition, the older cattle (> 4 years) showed significantly ($p = 0.01$) higher prevalence in comparison with cattle less than 4 years (Table 2). No statistically significant difference on BLV seroprevalence was noted between pregnant and non-pregnant cattle. Cattle originating from herd size more than 200 showed higher seroprevalence compared with those from herd lower than 200 and the difference was statistically non-significant ($p = 0.2$).

Regarding to age, pregnancy state and herd size found to be associated with high risk of BLV infection in buffaloes. The seroprevalence of BLV was significantly higher in older buffaloes (> 4 years), pregnant animals, and in large herd size, as in Table 3.

Discussion

BLV is mainly horizontally transmitted through direct exposures to biological fluids of infected animals such as blood and milk. After infection, the immune response in infected animal develops antibodies against envelope protein gp51 which can be detected serologically by ELISA (Nekoei et al. 2015).

Naturally, the disease occurs only in cattle but some previous studies reported BLV antibodies in buffaloes (Meas et al. 2000). Besides, the data about BLV infection in camel is very limited (Saidi et al. 2018).

The prevalence of BLV infection in cattle and buffaloes differed significantly between localities under the study. This may be due to different geographical regions and different management practices.

Table 1 Seroprevalence of BLV in cattle, buffaloes, and camels

Governorates	Species						*p* value
	Cattle		Buffalo		Camel		
	No. of positive	% of positive	No. of positive	% of positive	No. of positive	% of positive	
Kafr el-Sheikh	65/200	32.5	8/90	10	0	0	0.0001*
Qalyubia	5/100	5	0/10	0	0	0	
Menofia	3/50	6	0	0	0/100	0	
Total	73/350	20.8	9/100	9	0/100	0	

*The results are significant at $p < 0.05$

The results showed presence of antibodies against BLV in cattle (20.8%) and buffaloes (9%) but no antibodies against BLV were detected in camels based on ELISA test.

According to previous prevalence rate reported by Zaher and Ahmed (2014), it was 15.83%; the prevalence of BLV infections is growing. Also, the obtained results agree with a similar study which reported the prevalence rate of BLV infection in cattle 25.4% (Mousavi et al. 2014).

In another side, the obtained findings of BLV in buffaloes was slightly higher than those reported by (Meas et al. 2000), was 0.8%. This seroepidemiologic survey provides evidence that BLV infection is widespread around the world, and that not only dairy and beef cattle but also water buffaloes were infected. However, the occurrence of natural transmission of BLV is unknown (Meas et al. 2000).

In Egypt, the use of the same needle during treatment or vaccination in the same herd and use the same gloves in rectal palpation can play an important role in the horizontal transmission of BLV infection (Nekoei et al. 2015; Elhaig et al. 2017).

The high seroprevalence reported during the present study may be attributed to poor management factors and non-existent control programs for the disease. Also, the importation of unscreened heifers or frozen semen containing BLV for artificial insemination can increase the spreading of infection (Khamesipour et al. 2013; Selim et al. 2014).

On the other hand, we did not detect antibodies against BLV in camels, which maybe have a host resistant to BLV infections as previously argued by (Nekoei et al. 2015).

In support to the previous observation (Morovati et al. 2012; Mousavi et al. 2014), older animals had higher significant prevalence than younger animals. The higher prevalence in older animals is probably due to long exposure time. Contrary to expectations, no significant difference in seroprevalence was noted between pregnant and non-pregnant cattle and this comes in accordance with Morovati et al. (2012) and Selim et al. (2014). The obtained result could be attributed to the fact that the disease is mainly transmitted horizontally.

On the other side, the results of current study showed that the age, pregnancy state, and herd size have potential effect on BLV infection in buffaloes. The results come in accordance with Meas et al. (2000).

The obtained results may be attributed to the long life span of buffaloes allowing a longer period for BLV exposure which likely leads to high prevalence of BLV infection in buffaloes. Also, the pregnancy acts as stress factor on the animal, affecting on immune status which gives chance to BLV infection.

Our results revealed that the BLV infection is more prevalent in herd with more than 200 cattle or buffaloes. The intensive dairy production is usually depending on loose housing system which allows direct physical contact and increases the chance of horizontal transmission of BLV infection (Wu et al. 1989).

Table 2 The seroprevalence of BLV in cattle according to age, pregnancy, and herd size

Category	Level	Number tested	Positive	% seroprevalence	*p* value
Age group	< 4	100	13	13	0.012*
	> 4	250	60	24	
Pregnancy	Pregnant	270	61	22.9	0.09**
	Non-pregnant	80	12	15	
Herd size	< 200	100	35	35	0.2**
	> 200	250	38	15.2	

*The result is significant at $p < 0.05$

**The results are non-significant at $p > 0.05$

Table 3 The seroprevalence of BLV in buffalo according to age, pregnancy, and herd size

Category	Level	Number tested	Positive	% seroprevalence	*p* value
Age group	< 4	60	2	3.3	0.02*
	> 4	40	7	17.5	
Pregnancy	Pregnant	55	8	14.5	0.03*
	Non-pregnant	45	1	2.2	
Herd size	< 200	25	6	24	0.003*
	> 200	75	3	4	

*The results are significant at $p < 0.05$

The results of the current study revealed that antibodies are present in cattle and buffaloes and thus it seems necessary to implement control program to prevent the spread of BLV infection between animals. Further studies are required to investigate the economic impact and epidemiology of this disease in dairy cattle in the country.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethics statement The study was conducted according to the principles of good practice and approved by the ethical committee for animal experiments of the Faculty of Veterinary Medicine, Benha University.

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