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Seroprevalence and Risk Factors Assessment of Bovine Leukemia Virus in Cattle in Beheira, Egypt

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Abstract

Bovine leukemia virus (BLV) is the etiological agent of enzootic bovine leucosis (EBL), which is the most prevalent neoplastic disease of cattle worldwide. Few studies have been conducted on BLV detection in Egypt, and it is unknown whether BLV is prevalent in some areas. BLV seroprevalence has never been identified in Beheira province. Therefore, the main objective of this research was to determine the seroprevalence of BLV among cattle in Beheira. A total of 368 cattle plasma samples (219 dairy and 149 beef) from 6 dairy farms, 1 beef farm, and 9 slaughterhouses in eight districts covering most geographical areas of Beheira were investigated using a commercial ELISA for the detection of anti-gp51 antibodies. Data were analyzed, and the risk factors associated with BLV infection were evaluated. Out of the whole samples, 44 (11.9%) tested positive for BLV, and the seroprevalence rates in dairy and beef cattle were 31/219 (14.2%) and 13/149 (8.7%), respectively. Cattle breed had a significant risk factor on BLV seroprevalence, as in Holstein cattle, it was 21.65% (OR= 3.1, $P < 0.004$) higher than mixed local breed (8.20%) in dairy cattle. However, Colombian cattle showed the highest seroprevalence (19.15%) among tested beef cattle breeds. Additionally, neither age nor farming system had a potential risk on BLV seroprevalence in the tested dairy or beef cattle ($P > 0.1$). It is concluded that BLV infection is widespread among cattle in Beheira province's various localities, with a potential risk for cattle of foreign breeds to contract the BLV infection.

KEYWORDS
Bovine leukemia virus, Seroprevalence, Dairy, Beef, Cattle, Beheira, Egypt

INTRODUCTION

Bovine leukemia virus (BLV) is an oncogenic virus belonging to the family Retroviridae. It is the etiological agent of enzootic bovine leucosis (EBL), which is the most prevalent neoplastic disease of cattle (Gillet *et al*., 2007; Aida *et al*., 2013). Once infected, the animal becomes seropositive after three weeks and remains persistently infected lifelong (Tajima *et al*., 1998; Nagy *et al*., 2007). Approximately 65% of infected cattle remain clinically normal, 30% develop persistent lymphocytosis and 5-10% develop lymphosarcoma (Radostits *et al*., 2007). BLV infection commonly impairs the cattle industry worldwide and causes considerable economic losses due to early death of animals from lymphomas, carcass rejection at slaughter, reduction in milk supply, lower immunity, as well as effects on reproductive performance and longevity (Berg *et al*., 2015; Mekata *et al*., 2015; Marawan *et al*., 2021). BLV can be transmitted vertically and horizontally in addition to the other iatrogenic procedures involving the transfer of infected blood between animals (i.e., dehorning, ear tattooing, rectal palpation, and needle reuse), and is responsible for disease propagation in a herd (Hopkins and DiGiacomo, 1997). Although BLV-associated lymphosarcoma can affect both beef and dairy cattle, the disease more frequently occurs in dairy herds (Juliarena *et al*., 2017).

The World Organization for Animal Health (WOAH) has classified EBL as one of the diseases that could significantly affect global trade. Through the trade in breeding animals, BLV has invaded every continent and is currently a common disease of cattle worldwide (Polat *et al*., 2017). The prevalence of BLV in the cattle population varies greatly between different countries, ranging from (30-94%) in the United States (US), Asia, and South America (Scott *et al*., 2006; Murakami *et al*., 2013; Polat *et al*., 2016; More *et al*., 2017; LaDronka *et al*., 2018; Yang *et al*., 2019; Moe *et al*., 2020). On the other hand, the majority of the European nations have already reached the desired prevalence level (0.2% infected herds) or are on their way to achieve officially free status: meanwhile, the disease is still present in Romania, Bulgaria (Sandev *et al*., 2015), Hungary, Croatia, and Estonia, Greece, Poland, Italy and Portugal (Berg *et al*., 2015). In Africa, BLV infection has been reported in Botswana, Namibia, Zambia, Tanzania, and South Africa (Mushi *et al*., 1990; Kaura and Hübschle, 1994; Schoepf *et al*., 1997; Phiri *et al*., 2019; Suzuki *et al*., 2020). In Egypt, EBL cases were first diagnosed in 1997 in an imported cattle herd (Zaghawa *et al*., 2002). The WOAH (previously: OIE) considers that Egypt has been free from BLV since 1997 (Polat *et al*., 2017; Hamada *et al*., 2020). Recently, BLV has been reported in few provinces by using

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serological and molecular methods (Zaher and Ahmed, 2014; Selim *et al*., 2019; Hamada *et al*., 2020; Metwally *et al*., 2020; Selim *et al*., 2021).

Effective control and eradication programs of BLV depend on the accurate diagnosis and prevalence data of the disease. Therefore, a wide variety of techniques have been developed for BLV diagnosis in different laboratories globally, depending on the molecular methods to detect the integrated proviral genomic material in host genome by PCR (Tajima and Aida, 2005; Takeshima *et al*., 2016) and/ or other serological detection of anti-BLV antibodies in animal serum, plasma, and milk (Kurdi *et al*., 1999; Ladronka *et al*., 2018). A commercially available ELISAs are the highly sensitive and easily implemented procedure, and can be used to detect anti-BLV antibodies in clinical samples (Naif *et al*., 1992; Polat *et al*., 2017). Preventive measures have been effective in reducing the clinical effects of BLV infection. It is still debatable whether this strategy, which is entirely based on strict sanitary regulations and culling of the infected animals from the herd, is effective. The success of this strategy in eradication of BLV in several European nations serves as evidence of its effectiveness (Gutiérrez *et al*., 2014).

Egypt comprises more than 18 million animals, of them about 5 million cattle heads (FAO stat, 2018), with the highest density (approximately 20% of the total cattle population of Egypt) presents in Beheira province, which is located in the Nile Delta, the northern corner of Egypt. These cattle are reared in intensive, semi-intensive, or smallholder systems (Abdi *et al*., 2020). However, the nation's livestock numbers are decreasing gradually as a result of the severe losses due to outbreaks of infectious diseases (FAO stat, 2020). Even though BLV is widespread around the world, epidemiological data on its prevalence in some regions are still unavailable. Detailed epidemiological information on the prevalence of BLV throughout Egypt is needed. Up to date, there are few studies regarding BLV prevalence in Egypt, furthermore, BLV detection in Beheira province has never been reported. Therefore, the aim of this study was to detect the seroprevalence of BLV in dairy and beef cattle in Behaira province and to assess the risk factors related to the infection.

MATERIALS AND METHODS

Ethical statement

Ethical approval for this study was obtained from the Institutional Committee of Ethics in the Faculty of Veterinary Medicine, Damanhour University, Egypt (No. DMU/VetINF-2019-/0146). Permissions were obtained from the animal's owners for voluntary participation in the study and where permissions were not granted. All methods for the study were carried out in accordance with the relevant guidelines and regulations. All experiments have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Farms and Animals

A total number of 368 heads of cattle were investigated in this study during the period from January 2022 to October 2022. Dairy cows (n=219) and beef males (n=146) were sampled from 16 farms and slaughterhouses classified as 6 dairy farms, 1 beef farm, and 9 slaughterhouses distributed in eight different districts covering most of the geographical area of Beheira province in northern Egypt namely: Nubariyah, Abu El Matamir, Edku, Abu Hummus, El Delengat, Kafr El Dawwar, Damanhour, and Hosh Eissa (Figure 1). The number of tested farms was variable between districts. The dairy herds were comprising female cattle, while, the beef herd was comprising males, and the ages ranged from 2 to 12 years. However, males that had been collected from slaughterhouses were of 2 years old. Samples were collected randomly in all farms and slaughterhouses and the specific records of the tested animals' breeds, ages, sexes, and farming systems were reported. Of note, about 10% of the animals were sampled from each farm.

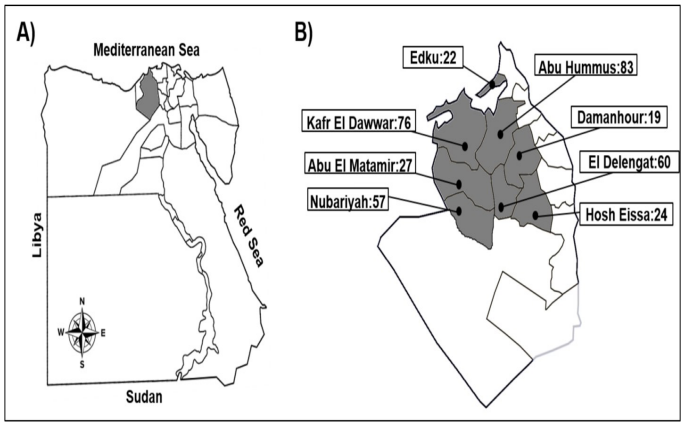

Fig. 1. (A) The geographical map of Egypt shows the location of Beheira province indicated by dark grey color. (B) The landscape shows the geographical distribution of the eight investigated localities in Beheira which are indicated by dark grey color and the numbers of tested samples from each district are written in boxes.

Blood sampling and plasma separation

Blood samples were collected for the serological examination of the presence of anti-BLV antibodies. Blood was drawn through the jugular vein in an aseptic manner, transferred to EDTA vacutainer tubes, and centrifuged for 15 minutes at 3000 rpm at room temperature to separate the plasma. Before testing, collected plasma were kept at -20°C.

Detection of anti-BLV antibodies by ELISA

All 368 plasma samples were serologically investigated for the presence of anti-BLV antibodies via competitive ELISA, using a commercial ELISA kit. Concisely, we used ID Screen Competitive ELISA kit for the detection of anti-gP51 antibodies in individual plasma samples (ID.vet, rue Louis Pasteur, Grabels- France). Steps of ELISA were done according to the manufacturer's instructions. The optical density (OD) was measured at 450 nm by an ELISA plate reader (Byonoy, Hamburg, Germany). The competition percentage (S/N %) of each sample was calculated as follows:

S/N =(OD sample)/(OD NC)×100

OD sample: mean optical density value of the sample, OD NC: mean OD value of negative control, and samples with an S/N value ≤ 50% were considered positive.

Statistical analysis

BLV seroprevalence in tested cattle was determined by counting the positive samples. The significance of the differences in the infection rates of the disease and risk factors were determined by the Chi-square (χ^2) test using online statistics software http://vassarstats.net/ and GraphPad Prism version 7. A P value of < 0.05 was considered statistically significant. Odds ratios (OR) at 95% confidence intervals were calculated using www.vassarstats.net and GraphPad Prism version 7.

RESULTS

Plasma samples of 368 cattle (dairy cows (n=219); beef calves (n=146)) from eight different districts in Beheira province (Figure 1) were investigated for the presence of anti-BLV antibodies. The total BLV seroprevalence was 44/368 (11.96%). Out of 219 dairy cows, 31 (14.16%); and of 149 beef males, 13 (8.72%) were seropositive for BLV antibodies (Table 1). The seroprevalence was significantly different among districts in case of dairy cattle (p <0.0001), but not in case of beef cattle breeds (p = 0.826) (Table 1). In details, El Delengat district had the greatest seroprevalence of BLV (10/23, 43.48%) among the examined dairy farms, followed by Nubariyah (20/57, 35.09%) and Abu Hummus (1/40, 2.50%). None of 27, 22, and 50 cows from Abu El Matamir, Edku, and Kafr El Dawwar dairy farms respectively, were tested positive (Table 1). Notably, anti-BLV antibodies were detected in beef cattle in all investigated localities. Indeed, Hosh Eissa had the highest BLV seroprevalence (3/24, 12.50%), while Damanhour showed the lowest (1/19, 5.26%). Other beef cattle plasma samples from Abu Hummus, El Delengat, and Kafr El Dawwar showed a BLV seroprevalence rates of (4/43, 9.30%), (2/37, 5.41%), and (3/26, 11.54), respectively (Table 1). The total BLV seroprevalence per region was calculated in the districts that were including both dairy and beef cattle. The highest seroprevalence was shown in El Delengat (12/60, 20.0%), followed by Abu Hummus (5/83, 6.02%), and Kafr El Dawwar (3/76, 3.95%) (Table 1).

In this study, an epidemiological investigation of risk variables was conducted to establish the effect of age, breed, locality, and farming system on the seroprevalence of BLV in both dairy and beef cattle. Risk variables were examined as potential risk factors for BLV positive cases, and the odds ratios and p value based on the Chi-squared test were calculated. Variable factors with OR >1

Table1. Seroprevalence of bovine leukemia virus in dairy and beef cattle from different localities in Beheira province.

Locality	Dairy cattle				Beef cattle				Total No. of farms & abattoirs	Positive / Total No (%)
	No. of samples	No. of positive (%)	95% CI	*P* value	No. of samples	No. of positive (%)	95% CI	*P* value		
Nubariyah	57	20 (35.09)	23.2 – 48.9	<0.0001	-	-		0.83	1	20 / 57 (35.09)
Abu El-Matamir	27	0 (0.00)	0 – 15.5		-	-			1	0 / 27 (0.00)
Edku	22	0 (0.00)	0 – 18.5		-	-			1	0 / 22 (0.00)
Abu Hummus	40	1 (2.50)	0.13 – 14.7		43	4 (9.30)	3.2 – 23.05		3	5 / 83 (6.02)
El-Delengat	23	10 (43.48)	23.9 – 65.1		37	2 (5.41)	0.94 – 19.5		3	12 / 60 (20.0)
Kafr El-Dawwar	50	0 (0.00)	0 – 8.88		26	3 (11.54)	3.02 – 31.3		3	3 / 76 (3.95)
Damanhour	-	-			19	1 (5.26)	0.27 – 28.1		2	1 / 19 (5.26)
Hosh Eissa	-	-			24	3 (12.50)	3.28 – 33.5		2	3 / 24 (12.50)
Total	219	31 (14.16)			149	13 (8.72)			16	44 / 368 (11.96)

Table 2. Risk factors associated with bovine leukemia virus infection among dairy cattle.

Tested variable	Positive / No of samples (%)	OR	CI % of OR		*P* value
			Lower	Upper	
Age					
< 4 years	0 /16 (0.00)	0	0	Infinity	0.56
4-6 years	27/155 (17.42)	2.3	0.8	6.4	0.16
> 6 years	4 /48 (8.30)	Ref.			
Breed					
Holstein	21 / 97 (21.65)	3.1	1.4	6.7	0.00
Mixed	10 / 122 (8.20)	Ref.			
Locality					
El-Delengat	10 / 23 (43.48)	30	3.9	332.2	0.00
Abu El-Matamir	0 / 27 (0.00)	0	0	Infinity	0.59
Edku	0 / 22 (0.00)	0	0	Infinity	0.64
Abu Hummus	1 / 40 (2.50)	Ref.			
Nubariyah	20 /57 (35.09)	21.1	2.7	165	0.00
Kafr El-Dawwar	0 / 50 (0.00)	0	0	Infinity	0.44
Farming system*					
Intensive	21 /147 (14.29)	1	0.5	2.3	0.9
Semi-intensive	10 /72 (13.89)	Ref.			

* Semi-intensive farm (10 -100 heads), intensive farm (100 -1000 heads).

were classified as susceptible to BLV infection, while those with OR <1 were not. Regarding the age of dairy cows, it was noticed that cows' group 4-6 years old had a higher seroprevalence of BLV (17.41%; ORs = 2.3; p = 0.16) than those of older than 6 years (8.31%) (reference group) or cows < 4 years old (0.0%; ORs = 0; p = 0.56), but that difference was not statistically significant (Table 2). In aspects of breeds of dairy cattle, it had been shown that the mixed breed had a seroprevalence rate of 8.20%, meanwhile, Holstein cows had a significantly higher seroprevalence (21.65%; OR = 3.1; P= 0.004) (Table 2). Notably, the seropositive cases of BLV had been detected only in three dairy farms out over the six examined districts. The lowest seroprevalence was reported in Abu Hummus (2.50%) which was used as a reference, thus a significant increase in seroprevalence was reported in El Delengat (43.48%; OR = 30; P= 0.0001), followed by Nubariyah (35.09%; OR = 21.1; P= 0.0001) in comparison (Table 2). For the dairy cattle farming system, there was no significant difference in seroprevalence rates between intensive (14.29%; OR = 1.0; P= 0.9) and semi-intensive farms (13.89%) (Table 2).

To examine the predisposing factors for BLV occurrence among beef cattle, the farming system, locality, and breed were considered. As all investigated beef calves were around two years old, age was ignored as a possible risk factor. A non-significant difference in BLV seroprevalence was noticed between the beef farm (15.38%; OR = 2.1; p= 0.37) and the slaughterhouses-originated samples (8.09%) (Table 3). The seroprevalence of BLV in Abu Hummus which was used as a reference was (9.30%). Anti-BLV antibodies were reported in all examined districts with non-significant differences whereas the seroprevalence rates were 12.50%, 5.41%, 11.54%, and 5.26%; ORs = 1.4, 0.6, 1.3, and 0.5; and p = 0.68, 0.68, 0.5, and 0.5 in Hosh Eissa, El Delengat, Kafr El-Dawwar, and Damanhour, respectively (Table 3). Additionally, for the beef cattle breeds, the mixed cattle breed (reference group) showed the lowest seroprevalence (2.25%), thus a significant increase was noticed in the Colombian calves (19.15%; OR = 10.3; p = 0.0006), followed by the Holstein calves (15.38%) but not significant (OR = 7.9; p = 0.07) in comparison (Table 3).

DISCUSSION

The Egyptian bovine sector is well integrated with cropland since there are limited natural pastures. Egypt continues to put policies in place to increase animal production. It is clear that an increase in cattle imports over the past ten years has boosted the performance of the cattle industry (FAO, 2018; Abdi *et al*., 2020). As Beheira province is the largest producer of agricultural crops needed for animal nutrition, it is comprising the highest number of cattle in entire the country. Therefore, this study was conducted to provide a sero-epidemiological survey on BLV infection among both dairy and beef cattle in such an important region of Egypt, with a focus on the risk factors related to its occurrence.

This study's overall BLV seroprevalence was 11.96% (44/368). To date, few studies have investigated the serological prevalence of BLV infection among cattle in north Egypt and this research is the first detection of BLV positive cases in Beheira province. Our previous report on the molecular detection of BLV in Egyptian cattle was the only investigated BLV in dairy farms in Beheira, however the number of samples was few and totally tested negative (Hamada *et al*., 2020). By comparing the BLV infection rate among the eight investigated districts in the province, the seropositive cases were reported in six regions (Nubariyah, Abu Hummus, El Delengat, Kafr El Dawwar, Damanhour, and Hosh Eissa) while cattle from Abu El Matamir and Edku were negative (Table 1). Additionally, the highest seroprevalence was in Nubariyah (35.09%) and the lowest was in Kafr El Dawwar (3.95%). These findings reported a higher total BLV seroprevalence in Beheira as well as in its examined districts than the previous seroprevalence rates of BLV in the neighbor regions and some other governorates in the Egyptian Delta such as Menofia (6.0%) and Qalyubia (5.0%) (Selim *et al*., 2019). This could be reasonable because the majority of cattle in Beheira were imported foreign breeds and reared in intensive farms of high density (Abdi *et al*., 2020; Metwally *et al*., 2020).

Noticeably, the BLV seroprevalence in the investigated dairy cattle was 14.16% with a significant increase than in beef cattle (8.72%). This could be reflective to the ages and breeds of the investigated cattle. As most of the dairy cows were females older than three years, while the beef calves were about two years old males and most of them were submitted to slaughter. Therefore, the dairy herds were able to maintain the BLV infection with a higher prevalence than beef cattle due to longevity and the possible vertical and horizontal transmission of the disease (Hopkins and DiGiacomo, 1997; Juliarena *et al*., 2017). In this study the reported BLV seroprevalence in dairy cattle was close to or lower than that reported in dairy cattle of some close geographical areas as Alexandria (20.3%), Kafr el-Sheikh (16.2%), Menofia (18.5%), and Qalyubia (17.1%) as determined by ELISA (Selim *et*

Table 3. Risk factors associated with bovine leukemia virus infection among beef cattle.

Tested variable	Positive / No of samples (%)	OR	CI % of OR		*P* value
			Lower	Upper	
Farming system					
Beef farm	2 /13 (15.38)	2.1	0.4	9.7	0.37
Slaughterhouses	11 /136 (8.09)	Ref.			
Locality					
Hosh Eissa	3 /24 (12.50)	1.4	0.3	5.6	0.68
Abu Hummus	4 / 43 (9.30)	Ref.			
El Delengat	2 / 37 (5.41)	0.6	0.1	3.2	0.68
Kafr El-Dawwar	3 / 26 (11.54)	1.3	0.3	6.2	0.5
Damanhour	1/ 19 (5.26)	0.5	0.06	5.2	0.5
Breed					
Colombian	9 / 47 (19.15)	10.3	2.44	48.5	0.00
Holstein	2 / 13 (15.38)	7.9	1	61.9	0.07
Mixed	2 / 89 (2.25)	Ref.			

*Age of all beef cattle ranged from 1.5- 2 years.

al., 2020), but higher than that in Alexandria (7.4%) as reported in an earlier serological study (Zaher and Ahmed, 2014).

The beef sector in Egypt is narrow scale, beef farms are limited, and is comprising male calves and infertile females for meat supply (Abdi *et al*., 2020). Therefore, the investigated beef samples were collected from nine slaughterhouses distributed in five regions and only one beef farm. The findings showed a decrease in BLV prevalence (8.72%) than our previous report that was conducted on the molecular detection of BLV among beef cattle (28.0%) in Egypt, in which we found 77.4% BLV prevalence in Cairo and 4.8% in Qena governorates (Metwally *et al*., 2020).

Egypt is importing the majority of its live dairy cattle from other countries like Germany, the Netherlands, and the US due to the low milk production of local cattle breeds. Egypt continues to rely on imported live beef cattle and frozen meat due to a shortage in beef cattle breeds and meat products. The primary sources of live beef cattle for Egypt include Sudan, Brazil, Spain, Colombia, Ukraine, Hungary, and India with the majority of these animals being destined for immediate slaughter (FAO, 2018; Abdi *et al*., 2020). The dairy cows included in this study were two breeds, local mixed breed and Holstein foreign breed. Interestingly, Holstein cows had a significantly higher seropositivity to BLV than mixed-breed cows. On the other hand, samples taken from beef calves were representing three different breeds named: Colombian, Mixed, and Holstein. The Colombian calves showed the highest BLV seroprevalence compared to the mixed or Holstein individuals. These findings indicate that the BLV is widely distributed among imported foreign breeds of cattle in Beheira province. This is consistent with previously reported data showing a 100% infection rate in one farm in the province of Damietta that comprised Holstein cows (Hamada *et al*., 2020). Additionally, in Assiut, upper Egypt, Zaghawa *et al*. in 2002, reported 50.3% of BLV infection following serological screening of a dairy herd of Holstein Friesian cattle imported from the US (Zaghawa *et al*., 2002). In contrast, Metwally *et al*. (2020) reported a BLV prevalence of 77.4% among beef calves of the mixed breed that were presented for slaughter in Cairo, which is a considerably higher rate than which we found among the mixed breed cattle in this study. This may be due to the random origin of the beef calves that were sold live either in livestock markets or to butchers or directly to slaughterhouses in Cairo, the largest and capital city of Egypt.

Loose housing in dairy farms and direct contact between calves and adult cattle in beef farms act as potential factors for the spread of BLV infection (Kobayashi *et al*., 2010). Consequently, BLV transmission is likely to be spread more in large-sized than small-sized herds as previously reported (Kobayashi *et al*., 2010; Haghparast *et al*., 2012; Selim *et al*., 2020). Contrary to expectations, there was no significant difference in the seroprevalence between the investigated semi-intensive or intensive farms in the investigated dairy population. Similarly, the findings revealed no discernible difference between samples obtained from slaughterhouses and beef farming herds. It seems unlikely that herd size alone would be a risk factor for higher within-herd BLV prevalence; instead, it appears that the risk association is caused by intermediary factors, like herd management techniques (Ladronka *et al*., 2018; Selim *et al*., 2021).

We found that dairy cows 4-6 years old had a higher seroprevalence than younger animals or elderly ones, supporting earlier observations (Erskine *et al*., 2012; Morovati *et al*., 2012; Selim *et al*., 2020). An increased seroprevalence in older cows in dairy herds as a result of the longer exposure time is likely linked. Moreover, cattle over 4-5 years old are more likely to develop the malignant form of B-cell lymphoma (Zaghawa *et al*., 2002; Radostits *et al*., 2007; Gutiérrez *et al*., 2014). Overall, the current study provided strong evidence that BLV infection is widespread among cattle in Beheira province's various localities, with a potential risk for cattle of the foreign breeds to contract the infection. Therefore, further research is needed to determine the risk of virus prevalence on large herd scales for the dairy and beef industries and to molecularly characterize the prevalent BLV genotypes in Beheira province.

CONCLUSION

The findings of this study presented new insights on the seroprevalence of BLV in dairy and beef cattle in Beheira province, Egypt. In this study, higher seroprevalence rates of BLV were reported in dairy cattle, and epidemiological risk for seropositivity in imported dairy and beef cattle of foreign breeds was also noticed. When these data combined with the fact that imported cattle breeds were kept in Egypt to create industrial cattle herds, there is a risk that the virus will spread throughout the entire population of cattle. To fully understand the wide prevalence of BLV infections in Egypt, further large-scale research is required. Additionally, the use of control strategies for prevention of the EBL occurrence in the Egyptian cattle farms is highly needed.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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