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Risk factor analysis of bovine leukemia virus infection in dairy cattle in Egypt

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ABSTRACT

Identification of the risk factors associated with Enzootic bovine leukosis (EBL) is essential for the adoption of potentially prevention strategies. Accordingly, our objectives were to determine the geographic distribution of Bovine Leukemia Virus (BLV) infection and identify the risk factors associated with cow-level BLV infection in the Egyptian dairy cattle. A cross-sectional study was conducted on 1299 mixed breed cows distributed over four provinces in the Nile Delta of Egypt in 2018. The randomly selected cows on each farm were serologically tested for BLV, and the cow's information was obtained from the farm records. Four variables (geographic location, herd size, number of parities, and age) were used for risk analysis. A total of 230 serum samples (17.7 %) were serologically positive for BLV. The highest prevalence of BLV infection was associated with parity (OR = 3.4, 95 %CI 2.4–4.9) with 80 % probability of being BLV-positive at parity ≥5, followed by herd size (OR = 1.8, 95 %CI 1.4–2.2). However, geographic location seems to have no impact on the prevalence of BLV infection in Egypt. Our findings strongly indicate that the intensive surveillance and effective prevention strategies against BLV infection in Egypt should be provided to multiparous cows with ≥5 parities and live in large farm with more than 200 cows.

1. Introduction

Enzootic bovine leukosis (EBL) is widely distributed contagious retroviral disease of cattle caused by Bovine Leukemia Virus (BLV). It is an endemic disease in many dairy herds with highly economic burdens [3,22]. It is a neoplastic disease, where the BLV infects lymphocytes and inserts its genomic material into the host's genome, thereby causing lifelong infections and most cases remain dormant with persistent lymphocytosis and increase in B-lymphocytes or B cells lymphomas in lymph node [21,24]. Approximately 30 % of the BLV infected animals have persistent lymphocytosis, with up to 5% developing B-cell lymphosarcoma, the most common neoplastic disease identified in slaughtered cattle in the United States [6,27,32,46]. Most infected cows do not display clinical signs and referred as asymptomatic or aleukemic [40].

Through developing tumors and negatively affecting the immune system of the infected cattle, BLV infection has deleterious effects on animal welfare and industry [3,12]. Enzootic bovine leukosis causes severe economic losses including premature death or culling of the infected animals and condemnation of carcasses after slaughter due to lymphosarcoma [29], where the control strategy of BLV infection should include test and segregation or test and slaughter [14,35]. In 2003, the economic losses of lymphosarcoma were estimated to be $412/case and the yearly direct losses associated with clinical BLV infections to the dairy industry is excess of $500 million [36]. In Japan, cattle with lymphosarcoma are not acceptable for human consumption [20]. Additionally, the USDA National Animal Health Monitoring System (NAHMS) in 1996 reported that the cows with BLV-test positive produced 218 kg less milk than the BLV-negative cows [9,31]. A negative association has also been reported between the herd-level milk production and herd BLV prevalence in Canadian dairy herds [39], Moreover, BLV infection has injurious impact on cow longevity [2], as well as restriction on the international trade of infected animals and its products between BLV-infected countries [33,38].

Typically, the viral particles are not free in the peripheral blood [18], but the provirus integrated in the lymphocytes of different body fluids, mostly milk and blood, results in increasing the infection time even with the presence of virus-neutralizing antibodies. Therefore, the

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seroprevalence is considered the substantial method to identify the source of BLV infection [1,25]. Enzootic bovine leukosis has been reported at different frequencies in many countries worldwide including United States, Japan and Argentina [16,30,31]. Approximately 40 % of dairy cattle in the United States are now infected with BLV [3]. Our search in the scientific databases revealed no published studies in peer-reviewed international journals investigated the BLV infection in Egypt (accessed on November 2019). The first BLV infection in Egypt was identified in imported dairy cows in Arab El-Aoumar, Assiut Province in 1996 [49]. In Kafr ElSheikh (KF), Alexandria (Alex), and Monofia (MF) provinces, the seroprevalence rate of BLV infections was 15.8 % among dairy herds [50]. Unfortunately, Egypt has not developed a national control program for BLV infection in dairy cattle. This is might because lack of reliable epidemiological data and risk factors analysis of BLV infection. The objective of our study was therefore to determine the seroprevalence of cow-level BLV infection over a broad geographic scale in Egypt and identify some of the risk factors associated with cow-level BLV infection in the Egyptian dairy cattle.

2. Materials and methods

Blood collections were performed under owner's consent, and the study was approved by the Internal Ethics Review Committee of Faculty of Veterinary Medicine, Benha University.

2.1. Study area and animals

Kafr elsheikh (KF), Alexandria (Alex), Menofia (MF), and Qalyubia (Qal) are the main provinces located in the north of Egypt. These provinces are located in the north climate zone which characterized by hot summer and cold winter. Adequate sample size for our study was calculated using Cochran's formula [11] as follow:

$$n = Z^2 \frac{p(1-p)}{e^2}$$

where n is the sample size, Z is the statistic corresponding to level of confidence, p is expected prevalence, and e is precision (corresponding to effect size). The level of confidence was used is 95 %, and the expected prevalence used in this study was 15 % based on the reported prevalence published in Zaher and Ahmed [50]. The precision (e) used in this study was 5 % based on [34].

During 2018, a total 1299 blood samples were collected from Holstein cattle raised in ten dairy herds located at four provinces in Egypt. The samples were categorized according to 4 provinces in the Nile Delta of Egypt (KF, Alex, MF, and Qal), age (ranged between 2–10 years old), number of calving (ranged between < 1, and > 6) and herd size (< 50, 50 − 99, 100 − 199, 200 − 299, and 300 − 400). The blood samples will be collected using evacuated blood collection tubes without EDTA for serum separation. The serum was separate by centrifugation at 3000 xg for 10 min and stored at − 20 °C until use.

2.2. Serological examination using ELISA

All serum samples were examined serologically using 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 sample 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.

2.3. Statistical analysis

Statistical analyses were performed using SAS 9.4 (SAS Inc., Cary, NC). The association of cow-level BLV seroprevalence with different risk factors was evaluated using the Cochran-Armitage trend test using PROC FREQ of SAS. The strength of association between risk factors and BLV seroprevalence was determined through Phi and Cramer's V value. Chi-square and stepwise forward multivariable logistic regression was used to identify the most important risk factor(s) associated with BLV infection. The *P*-values for entry into or removal from the logistic regression models were < 0.05. The logistic model, fitted with BLV infection as the outcome variable (present: 1, absent: 0), included fixed effects of the risk factors of parity (7 levels: 0, 1, 2, 3, 4, 5, and 6), age (5 levels: < 3, 4, 5, 6, and > 7 years), herd size (5 levels: < 50, 50 − 99, 100 − 199, 200 − 299, and 300 − 400), and geographic location (4 levels: KF, Alex, MF, and Qal). The stepwise elimination process was stopped once all remaining variables were significantly ($P < 0.05$) contributing to the model. The fit of the multivariable logistic regression model was assessed using the Hosmer–Lemeshow goodness-of-fit test. Number of parity and age were strongly correlated, and 2 multivariable logistic regression models were designed retaining either of these two variables. The model likelihood ratio $\chi 2$ and goodness-of-fit tests for both models were compared and number of parities was chosen over age as the variable to be retained in the final model due to a better performance of that model. A logistic regression model predicts the log odds (logit) for outcome as an additive function of the risk factors. The odds ratio was used as an approximate measure of relative risk (the likelihood of having BLV in animal with a given risk factor compared with animal without the risk factor). Confidence intervals for odds ratio estimates were obtained as described by Hosmer and Lemeshow [15]. Odds ratios greater than 1 indicate an increased risk of the outcome (BLV seroprevalence) with increasing value of risk factor and odds ratios less than 1 indicate a decreased risk of the outcome (BLV seroprevalence) with increasing value of risk factor [15].

3. Results

The seroprevalence of BLV was determined in 1299 serum samples obtained from Holstein cattle located in 4 provinces in the Nile Delta of Egypt at age ranged from 2 to 10 years. The distribution of cows based on risk factors was illustrated in Table 1.

The seroprevalence of BLV was not significantly differed between localities under the study. It was higher in Alex province (20.3 %), followed by MF (18.5 %), Qal (17.1 %) and KF (16.2 %) as shown in

Table 1
Descriptive analysis of variables used to predict the seroprevalence of bovine leukosis virus infection in dairy cattle of the Nile Delta of Egypt.

Variable	Category	No. of cows	Distribution (%)
Governate	KF	500	38.5
	Alex	300	23.1
	MF	200	15.4
	Qal	299	23.0
Herd size	< 50	75	5.8
	50 − 99	245	18.9
	100 − 199	295	22.7
	200 − 299	209	17.4
	300 − 400	371	28.6
Parity	0	169	13.0
	1	43	3.3
	2	362	27.9
	3	290	22.3
	4	48	3.7
	5	168	12.9
	6	219	16.9
Age (years)	< 3	183	14.1
	4	215	16.6
	5	456	35.2
	6	63	4.6
	> 7	382	29.5

Table 2
Univariable logistic regression analysis of the association of cow-level bovine leukosis virus infection with different risk factors in Egypt.

Variable	Category	No. of cows	No. positive	Prevalence (%)	*P*-value
Governate	KF	500	81	16.2	0.498
	Alex	300	61	20.3	
	MF	200	37	18.5	
	Qal	299	51	17.1	
Herd size	< 50	75	9	12	< 0.001
	50 – 99	245	21	8.6	
	100 – 199	295	19	6.4	
	200 – 299	209	50	23.9	
	300 – 400	371	131	35.3	
Parity	0	169	1	0.6	< 0.001
	1	43	1	2.3	
	2	362	7	1.9	
	3	290	39	13.5	
	4	48	10	20.8	
	5	168	69	41.1	
	6	219	103	47.0	
Age (years)	< 3	183	4	2.2	< 0.001
	4	215	8	3.7	
	5	456	63	13.8	
	6	63	22	37.3	
	> 7	382	133	34.8	

Table 2 and Fig. 1. The distribution of BLV-positive cows was differed according to the size of the herd ($P < 0.001$), and number of parities or age ($P < 0.001$; Table 2). The results of this study showed strong associations between the seroprevalence of BLV infection and parity (Phi Coefficient and Cramer's V = 0.56), and moderate association with age (0.47) and herd size (0.36). However, the Phi Coefficient and Cramer's V of 0.04 indicated no association between the BLV seroprevalence and geographic distribution.

The multiple logistic regression model indicated that parity and herd size were significant risk factors for BLV infected cows (Table 3). Multiparous cows had greater number odds for seropositivity of BLV (OR = 3.4, 95 % CI = 2.4 – 4.9), where the cows with parities ≥ 5 increased risk (80 %) of being seropositive for BLV (Fig. 2). High risk of BLV infection was also reported in the large herd size > 200 cows (OR = 1.8, 95 % CI = 1.4 – 2.2). However, geographic location was not associated with increased risk of being seropositive for BLV.

4. Discussion

Our study determines the cow-level seroprevalence and risk factors for BLV infection in dairy cattle located in the Nile Delta of Egypt. To the best of our knowledge, this is the first study to analyze the risk factors associated with BLV infection at cow-level on a broad scale in Egypt. The major advantages of the current study are: 1) broad context of many provinces, placing it amongst the few studies that have examined the prevalence of BLV in dairy cattle across the most densely dairy cattle populated areas on a national level; and 2) large number of participating cows from different herds size (1299 cows from 50 herds). The main finding of this study is that the parity ≥5 and herd size > 200 cows were the predominant risk factors for BLV infection in the dairy cattle in Egypt.

Bovine leukemia virus infections is widespread all over the world except in western Europe [37]. Most of surveys estimated the herd-level BLV infection and few studies are available that estimated the cow-level BLV infection in different countries. In Egypt, few studies reported BLV infection among dairy farms [1,49]. Overall, the cow-level seroprevalence of BLV infection of 17.7 % that reported here in this study is consistent with an earlier study that reported seroprevalence of 15.8 % in KF, Alex, and MF provinces [50]. In Canada, the cow-level seroprevalence of BVL infection was estimated at 20.8 % [29]. However, the animal-level prevalence of BLV has been increased in US from 39.6 to 68.7 % depending on the geographical region in 1996 to 83.9 % in 2007. In Argentina, the cow-level prevalence was 84 % [13,47]. The lower cow-level seroprevalence of BLV that reported in this study compared to high milk producing countries could partly be due to smaller herd size that might decrease the chance of animal exposure to virus [29]. Additionally, the milk production in Egypt depends, in considerable percentage, on smallholder dairy farmers.

Our study did not find significant associations between the BLV infection and the studied geographic regions in the Nile Delta of Egypt, the most densely dairy cattle populated area, that is consistent with earlier study [50]. This result is not surprising because the similar geographic nature of the land in the four studied provinces that is characterized by flat low-lying areas. Additionally, the Nile Delta has a stable dry and rarely rain climate condition. Consequently, we believe that the insect population density, the main transmission vector for BLV, are approximately similar in these provinces. However, further studies are required to evaluate that hypothesis and to examine finer scale climatic factors associated with insects' population dynamics in the Nile Delta of Egypt [44,45]. Moreover, dairy cattle trading between provinces is not common in Egypt. Several national-level studies didn't report significant difference in the prevalence of BLV between geographic regions [23,29], in agreement with general believe that the prevalence of BLV in endemic areas remains relatively steady over time [8].

In this study, we found that the herds size > 200 cows are associated with high prevalence of BLV in agreement with Baumgartener, Olson [4] that found increased within-herd prevalence of BLV in larger herds compared to small herds. Similarly, the 1996 and 2007 NAHMS and 2018 studies reported higher prevalence in larger herds. In Iran, it has been reported that the prevalence of BLV was higher in herds with more than 250 cows and a significant correlation has been also reported between the herd size and bulk tank milk antibodies against BLV [14]. Possible explanation for this finding is that the physical contacts between infected and uninfected animals is increased in larger herd resulting in increasing the chances of exposure to BLV infection. Paradoxically, no association has been reported between the herds size and the BLV prevalence in earlier studies [23,29]. This controversy is due to fact that the association between herd size and within-herd BLV prevalence is less easily explained and not consistent across all studies [23]. There is strong evidence that the herd size itself is not a risk factor for higher BLV prevalence but there are intermediates factors including management practices, which is different in herds of different sizes, are considered the true risk factors for higher BLV prevalence [23]. Therefore, further studies are required to determine and evaluate these intermediate factors.

At the cow-level, the highest positively association that was reported in this study was between the BLV seroprevalence and the parity. This finding is consistent with several earlier studies [9,10,23]. Cows with ≥4–5 parities are at higher risk of BLV infection compared to cows with less than 3 parities [48]. Increase the BLV prevalence in the multiparous cows or older cows may attributed to the chronic nature of the disease and longer age-associated duration of exposure to risk factors associated with the transmission of this disease, such as physical contact between infected and noninfected animals, use of common needles, and palpation sleeves [5,27].

A popular explanation of high prevalence rate is that the use of single needle for treatment and vaccination for large number of animals within herds [28,41,42]. Also, poor management, absence of control program over the country, importation of unknown status heifer from unscreened area or use infected semen in artificial insemination were possible causes for spreading of BLV infection between animals [19,43]

Furthermore, detection of BLV infection by ELISA testing is limited relative to actual viral load, where it has been reported that many younger cows with preexisting seronegative infections has been later

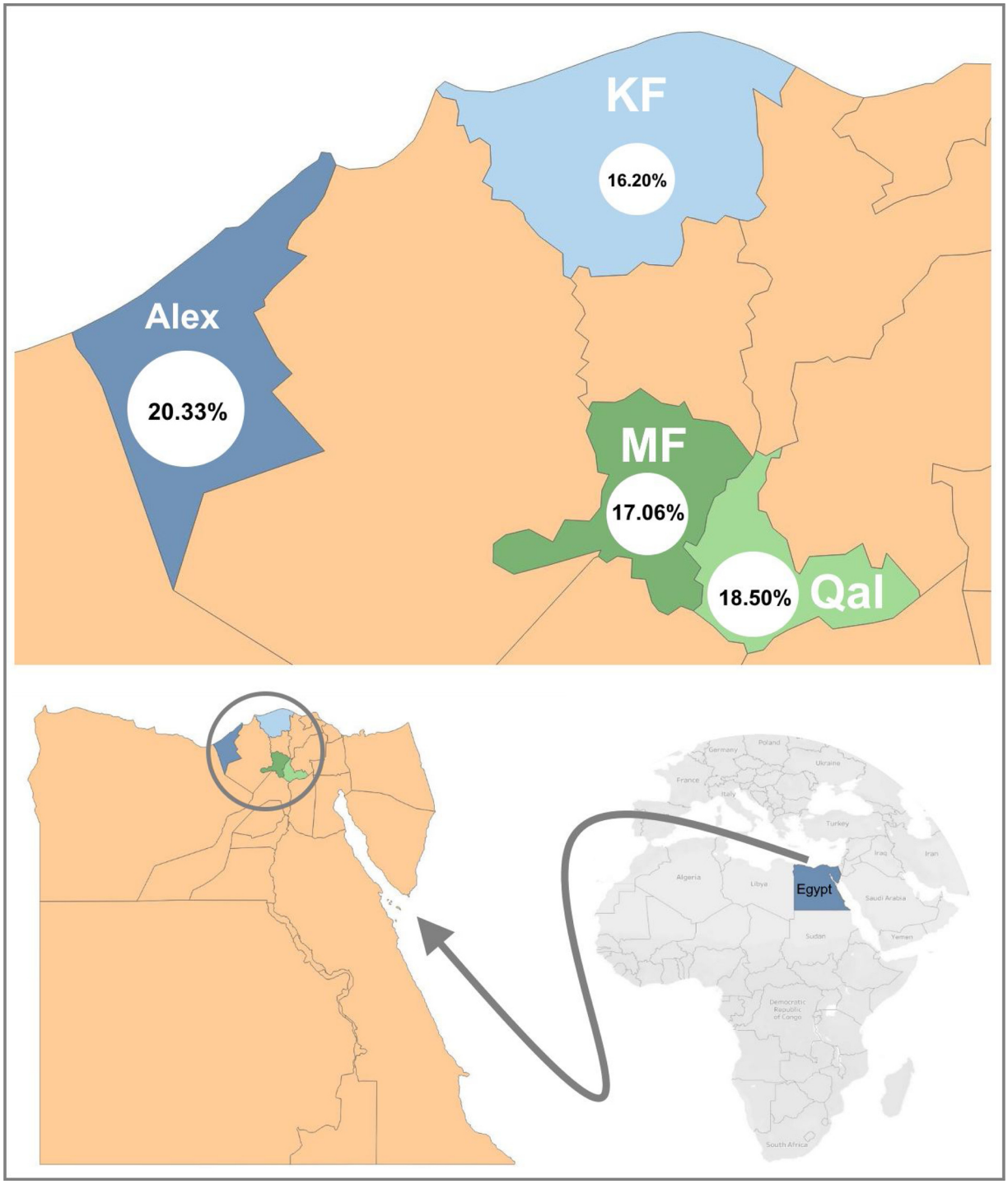

Fig. 1. Four sampling provinces (Kafr elsheikh, KF; Alexandria, Alex; Menofia, MF; Qalyubia, Qal) in in the Nile Delta of Egypt and the seroprevalence of bovine leukosis virus infection in each province.

Table 3
Multiple stepwise logistic regression analysis of variables associated with cows that are seropositive to leukosis in Egypt.

Variable	Estimated value	SE	*P*-value	OR	95 % CI_{OR}
Intercept	−4.39	0.73	< 0.001	–	–
Parity	1.22	0.19	< 0.001	3.4	2.4 − 4.9
Herd size	0.64	0.12	0.038	1.8	1.4 − 2.2
Governate	0.003	0.00	0.327	1.0	1.0 − 1.0

seroconvert to positive BLV infection without recent exposure to the BLV virus and this part of the age-associated increase in BLV seroprevalence [9]. Therefore; it seems that the age is both a main risk factor and an effect of BLV infection [9,26]. In the other hand, these findings may be attributed to few infected lymphocytes circulate in the low proviral load cattle which was not sufficient to induce detectable antibodies against BLV compared to high viral load cattle [6,17].

Recently, some study revealed relationship of some genetic variants with BLV infection where significant SNPs were associated with level of infection in BLV-infected cattle. Therefore, genome wide identification of genetics variants related with low proviral load BLV infections could

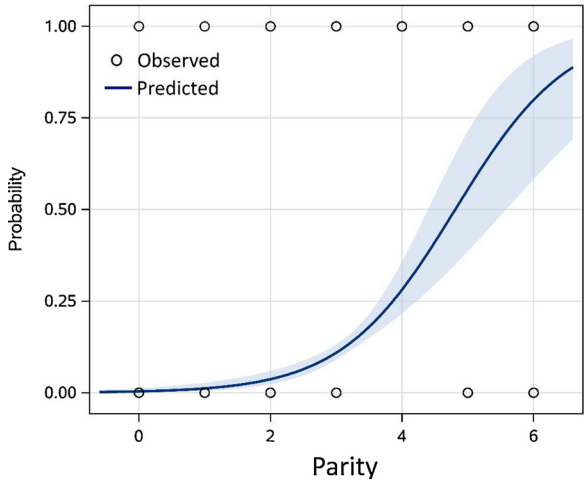

Fig. 2. Probability plot for the ability of parity to predict the seroprevalence of bovine leukosis virus (BLV) infection in the Nile Delta of Egypt. The curve shows the likely probability of seropositive (BLV) for each parity, with the 95 % shaded blue confidence interval.

be useful control programs based on selective management system via genomic selection in dairy cattle [7].

Two limitations of the present study warrant mention, 1) this study is a cross-sectional study that only able to evaluate association and longitudinal studies are therefore required to prove causation; 2) the ELISA testing used in this study has lower sensitivity and specificity than other ELISA kits for BLV which would influence the risk factor analysis in this study predictive values based on the BLV prevalence.

5. Conclusions

The results of this study showed that parity ≥5 and herd size > 200 cows seem to be among the main risk factors for BLV infection. Therefore, the present study helps in selection of animals that the control interventions may be most effective.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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