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The First Molecular Survey on Bovine Immunodeficiency Virus in Egyptian Cattle

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Abstract

Bovine viral immunodeficiency (BIV), often known as bovine lentivirus, is a disease affecting cattle and buffaloes worldwide. It is caused by the bovine immunodeficiency virus, a member of the *Retroviridae* family. Once BIV infects animals, it integrates the genome of the host cells and causes a progressive lifelong infection. Despite the record of the virus in various countries, BIV screening in Egyptian animals has never been investigated yet. Therefore, this study aimed to determine whether the BIV provirus was present in blood samples taken from dairy and beef cattle from six provinces in Egypt's northern, central, and southern regions. A total of 310 DNA samples extracted from cattle blood were investigated by the quantitative real-time polymerase chain reaction (qRT-PCR) technique using specific *pol* gene primers. The findings indicated the absence of BIV provirus in the analyzed samples. The cycle threshold (Ct) values of the tested samples range from 30 – 36.5, while the Ct values of negative control were 29.7 – 36.8. Because of the lack of positive control, UV light visualization of the target amplicon (385 bp) was conducted and revealed no evidence of BIV provirus in the tested samples. Although the investigated animals were grouped according to location, age, sex, breed, production type, and farming system, we could not analyze the potential risk factors because of the negative results in all tested samples. In conclusion, this study presented the first molecular screening of BIV provirus in Egyptian cattle. Additionally, a future large-scale survey on BIV in Egyptian cattle and buffaloes is recommended.

Keywords: Bovine immunodeficiency virus; Molecular screening; Cattle; Egypt

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1. Introduction

Bovine immunodeficiency virus (BIV) is a member of the *Retroviridae* family's *Orthoretrovirinae* subfamily of the *Lentivirus* genus, is closely related to human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) in the genomic structure and antigenic features (**Gonda et al., 1987; Adams et al., 2017**). Therefore, it is a study tool for other retroviruses using animals (**Garvey et al., 1990; Gonda et al., 1987**). BIV was first detected in the leucocytes of an 8-year-old dairy cow in Louisiana (USA) and was named R-29, which exhibited pronounced anorexia, sluggishness, neurological issues, and an elevated white blood cell count. Further examinations indicated perivascular infiltrates in the brain and widespread follicular hyperplasia in the lymph nodes (**Van Der Maaten et al., 1972**).

Evidence suggests that BIV, genetically related to the bovine leukemia virus (BLV), may inhibit the immune system, encourage secondary bacterial infections, and weaken the immunological response to immunizations (**Meas et al., 2000; Brujeni et al., 2010**). BIV has become distributed in many continents worldwide, including Europe, Asia, North America, and South America, and causes a progressive and persistent infection in cattle (**Gonda et al., 1987**). It can spread horizontally through the exchange of body fluids and blood or vertically through the colostrum and the placenta in the uterus (Moody et al., 2002). Most of the BIV-infected animals are asymptomatic; However, BIV-infected animals are variably associated with weight loss, secondary diseases, and decrease milk production **(González et al., 2008; Snider et al., 2003**). The BIV-infected cattle's hematological investigation revealed lymphocytosis and abnormal neutrophil and monocyte activity. Meningoencephalitis and skin lesions resistant to treatment have also been recorded in affected cattle.

The linear BIV genome has 8,960 base pairs and is made up of two copies of a single-stranded, positive-sense RNA that can bind to cellular DNA (**Gonda et al., 1987**). Similar to other lentiviruses, it contains the structural genes *gag, pol*, and *env*, flanked by two long terminal repeats (LTRs) on both sides which contain the promoters, enhancers, and terminators of BIV transcription and several non-structural accessory genes, including *vif* (viral infectivity factor), *tat* (transactivator of transcription), *rev* (regulator of virus expression), *vpw*, *vpy* and *tmx* that play an important role in the BIV replication and viral life cycle (**Avidan et al., 2006; Braun et al., 1988**). The BIV *Pol* region contains some conserved portions of the genomic structure which have been targeted in BIV molecular diagnosis. The most effective primers for detecting BIV in the experimentally infected bulls were the *pol* primers, which were discovered to be 30- to 100-fold more sensitive than the *env* primers (**Gradil et al., 1999**). Recently, some studies used the *pol* gene for BIV detection among cattle and buffaloes using the polymerase chain reaction (PCR) (**Keshavarz et al., 2022; Albernaz et al., 2015; Meas et al., 2004**).

BIV diagnosis is mostly based on finding BIV-specific antibodies by serology or the molecular detection of the viral genome through PCR (**Meas et al., 1998**). In Africa, BIV was detected only in Zambia (**Meas**

et al., 2004); however, the BIV prevalence rates were poorly investigated in other African countries. In Egypt, the BIV has not been investigated yet. Therefore, this study aimed to investigate the molecular prevalence of BIV among cattle from different geographic and climatic locations in Northern, Central, and Southern regions in Egypt. Moreover, cattle with different ages, sexes, breeds, production types, and from different farming systems were also targeted for BIV investigation.

2. Materials and methods

2.1. Ethical statement

The Animal Ethics Committee of the Faculty of Veterinary Medicine, Damanhour University, Damanhour, Egypt, set rules for handling all animals (Approval number, DMU/VetINF-2022-/0152).

2.2. Animal population and farms

A total number of 310 apparent healthy cattle (100 beef, and 210 dairy) from 24 cattle farms and four abattoirs located in six Egyptian provinces, scattered across Northern Egypt's Beheira and Damietta, Central Egypt's Cairo and Fayoum, and Southern Egypt's Qena and Luxor (**Table 1**). There were various types of cattle, including the Egyptian Native breed (n = 27), the Holstein breed (n = 83), and the Mixed breed (n = 200), which was a crossbreed between cattle from foreign breeds and Native cattle, and included cattle of both sexes (118 males and 192 females) of different ages, < 2 years (n= 39), 2-5 years (n= 113), and > 5 years (n= 158). Regarding farm size, a number of 127 cattle blood samples were obtained from 21 small-sized farms (< 200 heads per farm), 83 blood samples were collected from cattle in three large-sized farms (>200 heads per farm), and 100 blood samples were randomly collected from sporadic cases presented to slaughter in abattoirs.

2.3. Blood sampling and DNA extraction

Using the tail vein puncture technique, blood samples were drawn into a glass tube containing dipotassium ethylene diamine tetra-acetic acid (K2 EDTA) anticoagulant and then transported immediately into the laboratory. The Wizard Genomic DNA Purification Kit (Promega; Madison, WI, USA) was used to extract genomic DNA from 300 µl of whole blood in accordance with the manufacturer's instructions. Using the NanoDrop One Spectrophotometer, the concentration of DNA samples that had been extracted was determined (Thermo Fisher Scientific; Waltham, MA, USA). DNA samples were diluted in water free of nuclease for PCR tests to a final concentration of 100 ng/µl.

2.4. Detection of BIV provirus Using Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

qRT-PCR was used to identify BIV proviral DNA in the DNA samples by focusing on a conserved region of the BIV *pol* gene. A 385-bp fragment was amplified using the amplifying primers BIV-F (5'CCCTCCAGGAATTAAGGAATG3') and BIV-R (5'TCACTTTCTCTTCCTGGACCTT3'), as previously described (**Albernaz et al., 2015**). Briefly, the BIV *pol* region was amplified using an ABT 2X qPCR SYBR Green Mix according to the manufacture instructions (Applied Biotechnology, China) in a reaction mixture containing a total of 20 µl of 1 µl template DNA, 10 µl SYBR Green Mix, 1 µl containing 20 µM of each forward and reverse primers and 7 µl nuclease-free water. For negative control wells, we used 1µl nuclease-free water instead of the template DNA. The reaction was carried out in 96-well plates 0.1 ml (Applied Biosystems, Foster City, CA, USA), and the following heat profile was used for the PCR thermal cycles: denaturation at 95°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 1 minute. The final extension phase was given an extra 10 minutes after 40 cycles. The cycle threshold (Ct) values for each sample, as well as the negative control, were then reported. After electrophoresis on a 1.5% agarose gel stained with ethidium bromide, all amplified PCR products were visualized under UV light.

3. Results

BIV provirus was detected using qRT-PCR in DNA samples of cattle blood from different provinces representing the various Egyptian geographic and climatic regions. Cattle samples were collected from small and large farms or sporadic cases presented to slaughter in abattoirs, seeking more inclusive results and interpretation. The total BIV molecular prevalence in cattle from the six investigated Egyptian provinces was 0/310 (0.0 %) (**Table 2**). The complete absence of BIV provirus was recorded using the qRT-PCR as Ct values of the investigated DNA samples ranging from 30 – 36.5 Ct values compared to 29.7 – 36.8 Ct values in the wells of the negative control.

Because of the lack of positive control in the current study following agarose gel electrophoresis, the qRT-PCR results were seen using UV light. The results revealed the complete absence of DNA bands in all investigated PCR products and the negative control at the level of expected molecular weight (385-bp) as shown in (**Figure 1**).

Data in **Table 2** indicated the distribution and categorization of the investigated cattle from different provinces of Egypt, such as age, sex, breed, type of production, and farming system. The absence of BIV prevalence rates were reported in 115, 36, 38, 15, 90, and 16 DNA samples from Beheira, Damietta, Cairo, Fayoum, Qena, and Luxor provinces respectively. Regarding ages < 2 years, 2-5 years, and > 5 years' prevalence rates were 0/39, 0/113, and 0/158, respectively. Sex, breed, and production type did not show any positive samples among the investigated 118 males and 192 females, 27 natives, 83 Holstein, 200 mixed breeds and 100 beef and 210 dairy cattle. Neither large and small size farms nor sporadic cases showed positive samples among 83, 127, and 100 investigated animals, respectively.

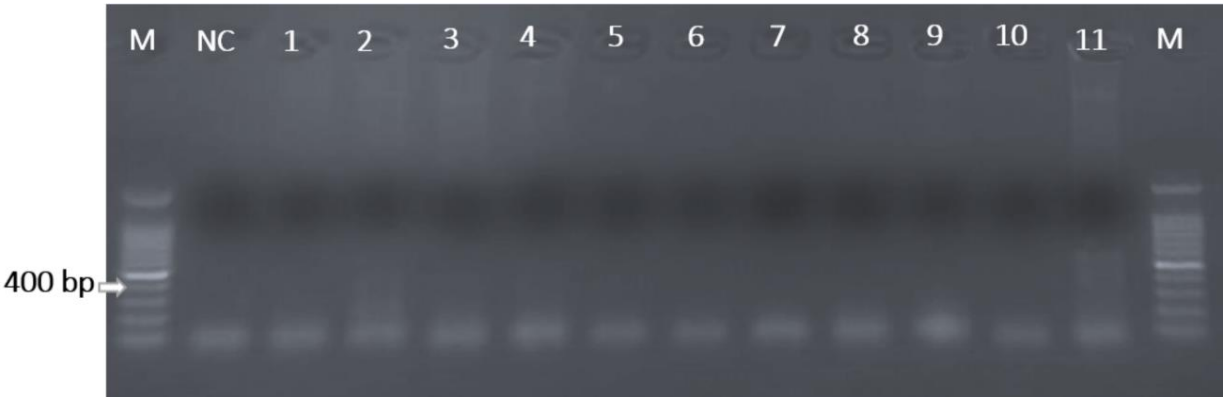

Figure 1. Polymerase chain reaction (PCR) products of BIV *pol* gene amplification of cattle DNA samples extracted from whole blood. The PCR products were visualized by UV light in a 1.5 % agarose gel stained with ethidium bromide after gel electrophoresis for 1 hour. M is molecular weight marker of 100 bp. NC is negative control. No. 1 – 11 are tested samples.

Table 1: Geographical locations and numbers of BIV-tested cattle from different provinces of Egypt

Population \ Province	Beheira	Damietta	Cairo	Fayoum	Qena	Luxor	Total
Geographical location	Northern	Northern	Central	Central	Southern	Southern	various
No. of tested farms / abattoirs	15 / 0	1 / 0	0 / 1	1/1	6 / 2	1 / 0	24 / 4
Farm size (Small / Large) *	14 / 1	0 / 1	0	1 / 0	5 /1	1 / 0	21 / 3
No. of tested samples	115	36	38	15	90	16	310

* Small-size farms (< 200 heads per farm), while large-size farms (> 200 heads per farm)

Table 2. Characteristics and prevalence of bovine immune deficiency virus in tested cattle

Factors & prevalence	No. of tested	No. of negative (%)	No. of positive (%)
Age			
< 2 years	39	39 (100.0)	0 (0.0)
2-5 years	113	113 (100.0)	0 (0.0)
> 5 years	158	158 (100.0)	0 (0.0)
Sex			
Male	118	118 (100.0)	0 (0.0)
Female	192	192 (100.0)	0 (0.0)
Breed			
Native	27	27 (100.0)	0 (0.0)
Mixed	200	200 (100.0)	0 (0.0)
Holstein	83	83 (100.0)	0 (0.0)
Production			
Beef	100	100 (100.0)	0 (0.0)
Dairy	210	210 (100.0)	0 (0.0)
Farm size			
Large size (> 200 head)	83	83 (100.0)	0 (0.0)
Small size (< 200 head)	127	127 (100.0)	0 (0.0)
Sporadic cases	100	100 (100.0)	0 (0.0)
Location			
Beheira	115	115 (100.0)	0 (0.0)
Damietta	36	36 (100.0)	0 (0.0)
Cairo	38	38 (100.0)	0 (0.0)
Fayoum	15	15 (100.0)	0 (0.0)
Qena	90	90 (100.0)	0 (0.0)
Luxor	16	16 (100.0)	0 (0.0)

4. Discussion

This study offered the first analysis of the existence of BIV provirus among the studied Egyptian cattle. The samples collected for this study were comprising 310 DNA samples extracted from cattle blood in 28 different production farms and sporadic cases represented for slaughter from abattoirs distributed across six Egyptian provinces. The animals under investigation represented several geographic and climatic regions of Egypt, including the Northern (Beheira and Damietta), Central (Cairo and Fayoum), and Southern (Qena and Luxor). Notably, the geographical location of Egypt is in two regions, North Africa, and Southwest Asia. It has an area of approximately one million $km^{2,}$ which is classified into 28 Egyptian provinces. Only about 5% of the total area is cultivated to provide crops and grains to about 100 million humans and 18 million animals including five million cattle (**FAO, 2020**).

Interestingly, after examining the extracted DNA samples using specific *pol* gene primers for BIV provirus amplification by the qRT-PCR technique, we found no evidence of BIV provirus among the investigated samples. The overall BIV prevalence rate was 0/310 (0.0%). Noticeably, the nested PCR (**Albernaz et al., 2015; Meas et al., 2004)** and one-step qRT-PCR (**Moody et al., 2002**) targeting the *pol* have a higher sensitivity than serology and virus isolation. Additionally, BIV testing by PCR is regarded as a trustworthy technique for finding infected cattle (**Suarez et al., 1998; Keshavarz et al., 2022; Albernaz et al., 2015; Meas et al., 2004**). For identifying proviral BIV DNA in mononuclear cells, sensitive PCR diagnostic techniques have been developed (**Zhang et al., 1997**). In our study, we correlated the Ct values of the tested samples and negative control with the visualization of the target PCR products to confirm the results of *pol* gene amplification because of the absence of a positive control sample. The Ct values of all investigated values were approximately similar to the negative control, and there were no Ct values less than 30. The absence of positive Ct values was confirmed by the complete lack of the target PCR bands during visualization of injected gel stained by ethidium bromide.

In contrast to our results, BIV has been recorded among cattle and buffaloes in many countries globally. Six BIV isolates were obtained in two countries worldwide (**Bhatia et al., 2013**). There were three isolates, FL491 and FL112, from Florida (Suarez et al., 1998) and R-29, a cow with persistent lymphocytosis (Van Der Maaten et al., 1972), in the United States. The other three isolates were BIVCR1 from Costa Rica (**Hidalgo et al., 1995**). BIV among dairy and beef cattle and buffaloes has been detected by molecular and serological methods in the United States with a prevalence of 21% (**Cockerell et al., 1992**), Canada (5.5%) (**McNab et al., 1994**), Mexico (28.9%) (**González-Fernández et al., 2020**), Germany (6.6%) (**Muluneh, 1994**), France

(3.8%) (**Polack et al., 1996**), Switzerland (**Gene, 1994**), Australia (17%) (**Forman et al., 1992**), the United Kingdom (**Clayton, 1994**), Italy (2.5%) (**Cavirani et al., 1998**), Japan (7.5%) (**Hirai et al., 1996; Meas et al., 1998**), Korea (33%) **(Cho et al., 1999)**, Iran (9.1%) (**Brujeni et al., 2010; Bhatia et al., 2006; Tajbakhsh et al., 2010; Keshavarz et al, 2022**), India from 10 blood samples (**Patil et al., 2003**) and Brazil (4.4%, and 12.5%) (**Meas et al., 2002; Albernaz et al., 2015; Rodrigues et al., 2019**). However, Zambia is the only African country that reported BIV in cattle, with a prevalence of 11.4% (**Meas et al., 2004)**.

Although Egypt imports much of its live dairy and beef cattle from BIV-endemic countries, surprisingly, our results did not report any positive samples among native and imported cattle breeds. Dairy cattle usually are imported from Germany, the Netherlands, and the United States 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. Egypt's highest sources of live beef cattle are Sudan, Brazil, Spain, Colombia, Ukraine, Hungary, and India **(FAO, 2018; Abdi et al., 2020)** where BIV was also recorded. Most Egyptian cattle are reared for dairy purposes however, male calves and infertile females are reared for beef production in intensive, semi-intensive, and smallholder farming systems (**Abdi et al., 2020**). Our results did not show the influence of the risk factors such as location, age, sex, breed, production type, and farming system on the BIV prevalence rate because of the negative results in all categorized cattle. In this context, Egypt's climate is suitable for insect-borne diseases such as BIV, as in the northern and middle regions, where summers are hot with moderate humidity and winters are mildly cold and rainy along the coasts; meanwhile, the climate in the southern regions is very dry, hot, and rarely rains. Furthermore, the presence of several water canals along the sides of the Nile River aids in the propagation of insect populations. In contrast, another study in Brazil reported the effect of the farming system as a risk factor. Management techniques used in the infected farms may cause the BIV prevalence and greater infection rate (**Albernaz et al., 2015**). Vaccinations, deworming, pregnancy detection, blood collection for brucellosis and tuberculin tests, and other procedures frequently require the animals to be brought to the pen, despite the fact that they have been grown extensively. As a result, the animals pack together. Animals' bodily fluid exchanges can facilitate the BIV horizontal transmission. Additionally, given those animals frequently share needles and other contaminants, BIV transmission may happen through the iatrogenic pathway (**Moody et al., 2002**).

5. Conclusion

This study provided Egypt's first molecular screening of BIV prevalence among dairy and beef cattle. The BIV provirus was investigated by qRT-PCR a technique using specific *pol* gene primers. The results of this study indicated the absence of the BIV provirus among the examined animals from different geographical locations in the Northern, Central, and Southern six provinces of Egypt. Although cattle samples were classified into different groups, the influence of risk factors such as location, age, sex, breed, production type, and farming system on the BIV prevalence could not be analyzed due to the negative results. We recommend a further large-scale prevalence survey on BIV infections among cattle and buffaloes in Egypt.

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Authors contributions

Conceptualization and design; SM, RH., Experiments, formal analysis, investigation; SM, RF, BE, RH; Resources and shared materials; SM, RF, BE, RH., Writing—original draft, SM, RH; Writing—review and editing; SM, RF.

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Conflict of interests

The authors declare that they have no conflict of interest.

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