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REGULAR ARTICLES

Seroprevalence of Rift Valley Fever virus in one-humped camels (*Camelus dromedaries*) in Egypt

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Abstract

Rift Valley fever (RVF) is a mosquito-borne viral disease that affects a variety of domestic animals, including cattle, sheep, goats, and camels, and has zoonotic potential. Although the rift valley fever virus (RVFV) is usually asymptomatic in camels, it can induce abortion in some pregnant animals. In the current study, a serosurvey was carried out to investigate the prevalence of RVFV antibodies and related risk factors in camels from four Egyptian governorates. A total of 400 serum samples were examined for anti-RVFV antibodies using a competitive enzyme-linked immunosorbent assay (c-ELISA). The results revealed that the overall prevalence of RVF among examined camels was 21.5% and the disease was more prevalent in Kafr ElSheikh governorate in Nile Delta of Egypt. In addition, the age group of camels with more than 5 years (OR=4.49, 95%CI: 1.39–14.49), the female sex (OR=3.38, 95%CI: 1.51–7.58), the emaciated animals (OR=1.52, 95%CI: 0.86–2.66), the summer season's infection (OR=5.98, 95%CI: 1.79–19.93), the presence of mosquitoes (OR= 2.88, 95%CI: 1.39–5.95), and the absence of mosquitoes control (OR=3.97, 95%CI: 2.09–7.57) were identified as risk factors for RVFV infection. The results of this study support knowledge on the risk factors for RVFV infection and demonstrate that camels raising in Egypt have RVFV antibodies. Quarantine measures or vaccination program should be implemented to reduce the likelihood of RVFV introduction, dissemination among susceptible animals, and ultimately transmission to humans.

Keywords Seroepidemiology · Rift valley fever · ELISA · Risk factors · Camels · Egypt

Introduction

Rift Valley fever (RVF), is an insect-borne disease, is mostly common in some regions of sub-Saharan Africa, where virus activity ranges from a mild enzootic to large outbreaks that have occurred in numerous areas (Aradaib et al. 2013). The disease is caused by Rift Valley fever virus (RVFV), which belongs to *Phlebovirus* genus and *Phenuiviridae* family (Ly et al. 2017).

Aedes and *Culex* mosquitoes and other haematophagous arthropods are the main vectors for the transmission of RVFV through bites (Baz et al. 2022a; Melaun et al. 2014; Radwan et al. 2022a; Radwan et al. 2022b; Selim et al.

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2019; Selim et al. 2020c). It can be contracted by intimate contact with contaminated animal materials and fluids as a result of an occupational hazard (Pepin et al. 2010; Talavera et al. 2018). Moreover, other possible transmission pathways, including as migratory or wind-borne vectors, the movement or importation of viraemic animals, or the transfer of infected persons or vectors by aircraft, also play a part in RVFV transmission (Linthicum et al. 2016).

The disease's epidemiology is highly complicated and involves a number of participants, including humans, domesticated livestock, wild animals, and mosquitoes (Bukbuk et al. 2014). Cattle, sheep, goats, and camels are highly vulnerable to RVF and act as the virus's amplifying hosts. Usually, outbreaks of the disease are caused by a high density of mosquito vectors (Linthicum et al. 2016). The virus is mostly kept alive during the inter-epidemic period by circulating at low levels in livestock, wildlife, and people and being spread by mosquitoes (Kariuki Njenga and Bett 2019).

RVF is usually endemic in Sub-Saharan Africa and has been recorded in several African countries, including Uganda, Kenya, South Africa, and Sudan. This has a negative impact on the socioeconomic well-being of the populations impacted (Mansfield et al. 2015; Rich and Wanyoike 2010). Nonetheless, RVFV periodically spreads to other regions, it was reported at northward of Egypt in 1977 and eastward over the Red Sea into Saudi Arabia and Yemen in 2000 (El-Rahim et al. 1999; Madani et al. 2003; Shoemaker et al. 2002). Moreover, RVF outbreaks involving ruminants and human have also occurred on the coasts of Africa and the Arabian Peninsula (Balkhy and Memish 2003). In 1959, Nigeria reported the first case of RVF in Merino sheep, which imported from South Africa (Ferguson 1959). Also, it has successfully isolated RVFV from *Culex antennatus* and *Culicoides* (Lee 1979).

Agricultural communities can suffer greatly from RVF in livestock, which causes high mortalities in young animals and significant rates of abortion in adult animals (Al-Afaleq et al. 2012). In contrast, RVFV can cause a mild febrile disease in humans but is otherwise asymptomatic. Only a small percentage of cases (1-2%) lead to more serious illnesses as encephalitis, acute hepatitis, retinitis, and/or hemorrhagic syndrome (Adam et al. 2010; Meegan and Bailey 2019).

The camel (*Camelus dromedarius*), which is particularly adapted to hot and arid environments, is referred to as the ship of the desert. Since it may be used for transportation, racing, and tourist attractions as well as a source of meat and milk, it is significant commercially in Egypt (Borham et al. 2017; Selim et al. 2022a). In Egypt, camel calves might be fattened using the intense feeding program, which would address the issue of a lack of red meat. Its meat is also high in protein and iron, low in fat and cholesterol, and a healthy choice due to its therapeutic characteristics (El-Badawi 2018).

Camels are most frequently seen in Egypt's semi-arid and desert regions. They promote the production of high-quality meat and milk, and they facilitate efficient agricultural services (Selim and Abdelhady 2020b; Selim et al. 2022a; Selim and Ali 2020; Selim et al. 2022b). Initially, it was believed that they would be resistant to the majority of pathogens that affect domestic animals. However, recent studies have shown that they are susceptible to a variety of infectious diseases, including some that have the potential to spread to other people, like RVF (Britch et al. 2013; El Harrak et al. 2011).

The impact of RVF on livestock sectors and producers, as well as on global trade, may be significant. RVF may have a serious impact on public health, approximately 200,000 infected and 600 death cases recorded in the pandemic in 1977 in Egypt (Meegan 1979). In addition to that outbreak, RVF again struck Egypt in 1993, 1994, 1997, and ultimately in 2003 (Ahmed Kamal 2011). Recently, another study confirmed low level RVFV circulation in various locations of Egypt (Mroz et al. 2017). The continual importation of camels or viremic ruminants, mostly from Sudan, was thought to be the main entrance way for RVF into Egypt (Ahmed Kamal 2011). In addition, RVFV control is impeded by vector control issues and the fact that vaccination are only accessible for ruminants, employing either inactivated vaccines with limited efficiency or live attenuated vaccines with a pathogenic impact.

In Egypt, there is limited information available regarding the epidemiology of RVF among camels. Epidemiologic researches, including improved surveillance, are urgently needed to be able to forecast and respond to the RVF epidemics among camels in Egypt more accurately.

As a result, the purpose of this study was to determine the seroprevalence and possible risk factors for RVFV exposure in camels raised in four Egyptian governorates.

Materials and methods

Ethical statement

The Benha University ethics committee for animal studies approved all methods, including blood sample handling and collection. The animal owners gave their informed permission for the collecting of samples. The ARRIVE criteria were adhered to throughout the entire study process.

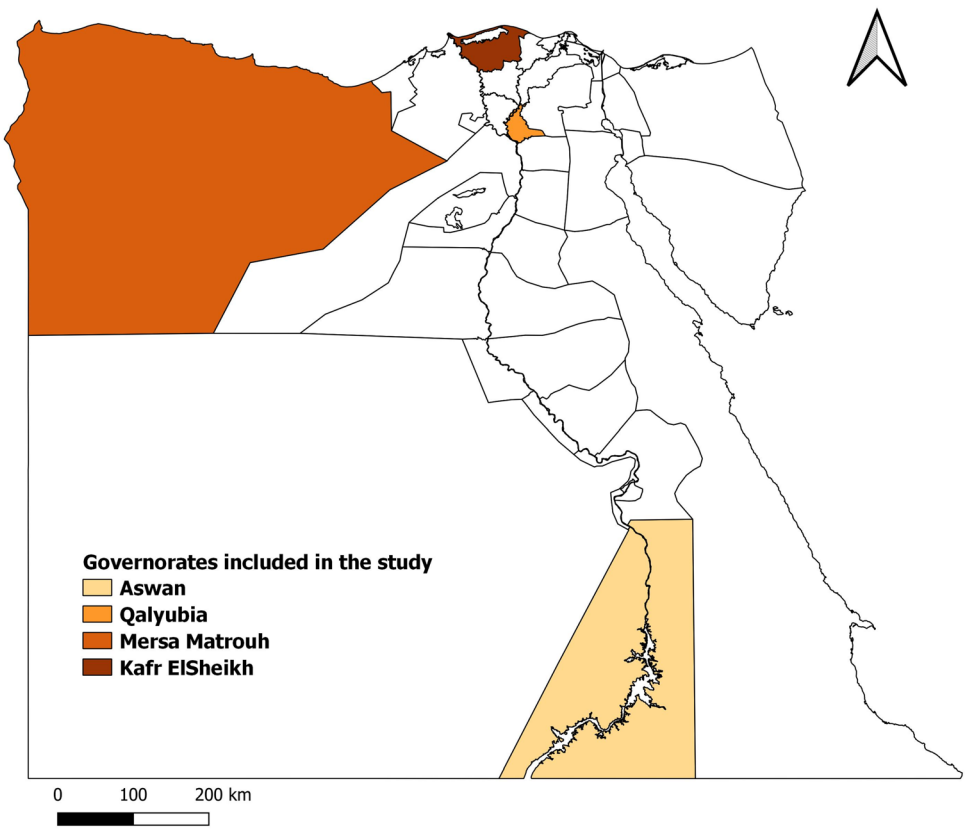

Fig. 1 MAP of Egypt showing the governorates involved in the study (MAP generated by QGIS software)

Study area

The study was carried out in four Egyptian governorates: Kafr ElSheikh and Qalyubia in the Nile Delta, Mersa Matrouh on the Mediterranean coast, and Aswan in the south, (Fig. 1). The four selected governorates have a large population of camels which used in agriculture field or in tourism. The delta region receives only 100 to 200 mm of rain per year and the most of it falls during the winter. The delta has its warmest weather in July and August, with a maximum average temperature of 34°C. While the temperature in winter time is typically low and vary from 9°C at night to 19°C during the day. On another side, Aswan located at Latitude 24° 5′ 23″. Aswan has a relatively dry climate all year, with less than 1 mm of precipitation on average. Aswan is one of the least humid cities on the earth, with an average relative humidity of about 26%, with a high mean of 42% in winter and a minimum mean of 16% in summer.

Study design and sample size

Between August 2021 and July 2022, a cross-sectional study was carried out to determine the seroprevalence of antibodies against RVFV in camels and assess the possible risk factors for the RVFV infection. The sample size was calculated using the following formula according to Thrusfield (2018).

$$n = \frac{z^2\ P(1-p)}{d^2}$$

Where n is the number of required sample, P is the expected prevalence which was 20.65% according to Mahmoud and Ali (2021), d is the absolute precision 5% with 95% confidence interval (Z=1.96). Hence, the calculated sample size was 245 serum samples. However, the sample size was increased to 400 to improve the accuracy of the estimation of the prevalence rate.

Sampling collection

The blood samples were taken from restraining camels in laying down position by help of two persons. Blood sample (5mL) was collected using random sampling method from the jugular vein using vacutainer tubes without anticoagulant that was labelled, then transported immediately in ice box to the Veterinary Diagnostic Laboratory at the Faculty of Veterinary Medicine at Benha University. For proper separation of the serum from clotted blood, the sample was centrifuged at 30.000 ×g for 10 min. We separated and harvested the

sera in the labelled 2 mL Eppendorf tubes, then kept them at -20°C for sample analysis.

Questionnaire

The data about the animals included in this study are obtained through a questionnaire filled out by their owners. The questionnaire included individual risk factors such as age (≤2 years, 2–5, ≥5 years old), sex (male, female), and emaciated or healthy body condition, as well as management risk factors such as herd size (small less than 50 camels or medium between 50 and 100 camels), season (September–November, June–August, December-February, March–May), mosquito vector (present or absent), mosquito control as regular application of insecticides every week (yes or no), and the four study governorates.

Serological analysis

The serum samples were examined serologically using a competitive Enzyme-Linked Immunosorbent Assay (ELISA) kits in order to identify anti-RVFV-Nucleoprotein antibodies in serum. The ID Screen® Rift Valley Fever Competition Multi-Species kit (Innovative Diagnostics, IDvet, France) was used in accordance with the manufacturer's instructions. In each well of the ELISA plate, 50 µL of the dilution buffer were added. Then, 50 µL from positive and negative control were added in two wells for each one and 50 µL from samples in the rest of the plate and incubated at 37°C for one hour. After that, the plate was emptied and wash three times by 300 µL of washing solution, then added 100 µL Anti-RVF-NP Conjugate 1X to each well and incubated at 21°C for 30 min. After incubation, repeat the washing step and added 100 µL substrate solution to each well, followed by incubation at 21°C for 15 min. Finally, 100µL of the stop solution added to each well, and read the plate at 450 nm using ELISA reader plate AMR100 (AllSheng, China).

A competition percentage (S/N%) for each sample was calculated using the formula S/N%= (OD450 nm_{Sample}/OD450 nm_{NC}) ×100.

Samples with a competition percentage (S/N%) of 40% or less were considered positive, while those with a competition percentage of 40 to 50% were labelled doubtful. Samples were considered negative if the competition percentage was greater than 50%.

Statistical analysis

The gathered information was entered onto a Microsoft Excel spread sheet in a computer. SPSS version 24 (IBM, SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. Using the Chi-square ($\chi 2$) test in a univariable analysis to determine the association between seropositivity of camels for RVF infection and its possible risk factors. We evaluated multicollinearity and eliminated highly collinear variables with a higher *P*-value. The significant variables in univariable model were entered into the multivariable logistic regression model. Significant data was defined as a *P*-value <0.05. The goodness-of-fit of the model was evaluated using the Hosmer-Lemeshow statistic.

Results

According to findings of the present study, the overall seroprevalence of RVF in camels from the four governorates was 21.5%. The seroprevalence was non-significant (*P*=0.074) differed between studied governorates, camels from the Kafr ElSheikh governorate had the greatest seropositivity (31.8%), followed by Mersa Matrouh, Qalyubia and Aswan with 20%, 18.9% and 13.3% respectively, Table 1.

The Chi-squared test revealed no significant relationship (*P*>0.05) between the herd size and seropositivity of the tested camels. The majority of examined camels raising in small herd showed higher seropositivity to RFV (24.3%) when compared to camels living in medium herd (15%), Table 1.

In addition, the seroprevalence of RVF in camels was significantly (*P*<0.05) associated with age, sex, season, mosquitoes presence and control. The seroprevalence was 10% (10/100) in males and 25.3% (76/300) in females. Whereas, camels older than five years had a prevalence of RVF of 30.7% (46/150), compared to those younger than two years (7.3%), Table 1.

According to season, the highest prevalence rate found in June–August (40%) and the lowest in the September–November (10%), while the medium level observed in the December-February (16.4%). The results confirm the findings about the role of mosquitoes in the spreading of the RVFV, where the seroprevalence of RVF was significantly (*P*<0.05) increased in presence of mosquitoes (27.4%) and in absence of mosquito control (29.4%), Table 1. From these results, it is clear that emaciated camels were more susceptible to RVF infection, the seroprevalence was 27.6% but it was 16.3% in healthy camels.

The results of multivariable logistic regression analysis for the significant variables presented in Table 2. When compared to young camels (under 2 years of age), older camels (>5 years of age) had a four-fold higher likelihood of having been infected with RVF (OR = 4.49, 95%CI = 1.39–14.49, *P*-value = 0.012). In addition, RVF infection was three times more likely in females than in males (OR = 3.38, 95%CI = 1.51–7.58, *P*-value = 0.003). The risk to get RVF increased during June-August months (OR = 5.98, 95%CI = 1.79–19.93, *P*-value = 0.004), especially in presence of mosquitoes (OR = 2.88, 95%CI = 1.39–5.95, *P*-value = 0.004) and absence of mosquitoes control application (OR = 3.97, 95%CI = 2.09–7.57, *P*-value <0.0001).

Table 1 Univariable analysis of the relationship between possible risk variables and RVF seropositivity in Egyptian camels

Variable	No of examined camels	No of positive	No of negative	% of positive	95%CI	Statistic
Locality						
Qalyubia	95	18	77	18.9	13.34-27.98	$\chi 2$=6.929 d=3 *P*=0.074
Kafr ElSheikh	110	35	75	31.8	23.86-41.01	
Mersa matrouh	105	21	84	20.0	13.47-28.65	
Aswan	90	12	78	13.3	7.79-21.87	
Age						
≤2	55	4	51	7.3	2.86-17.26	$\chi 2$=10.020 d=2*P*=0.007*
>2-5	195	36	159	18.5	13.64-2.45	
>5	150	46	104	30.7	23.85-38.46	
Sex						
Male	100	10	90	10.0	5.52-17.44	$\chi 2$=7.228 d=1*P*=0.007*
Female	300	76	224	25.3	20.74-30.54	
Herd size						
Small (less than 50)	280	68	212	24.3	19.64-29.64	$\chi 2$=2.864 d=1 *P*=0.091
Medium (50-100)	120	18	102	15.0	9.7-22.47	
Season						
September-November	40	4	36	10.0	3.96-23.05	$\chi 2$=21.591 d=3*P*<0.0001*
June-August	120	48	72	40.0	31.68-48.94	
December-February	110	18	92	16.4	10.61-24.38	
March-May	130	16	114	12.3	7.72-19.06	
Mosquitoes presence						
No	130	12	118	9.2	5.36-15.44	$\chi 2$=11.773 d=1 *P*=0.001*
Yes	270	74	196	27.4	22.43-33.02	
Mosquitoes control						
Yes	165	17	148	10.3	6.53-15.88	$\chi 2$=13.945 d=1*P*<0.0001*
No	235	69	166	29.4	23.91-35.48	
Body condition						
Emaciated	185	51	134	27.6	21.64-34.42	$\chi 2$=4.827 d=1*P*=0.028*
Healthy	215	35	180	16.3	11.94-21.8	
Total	400	86	314	21.5	17.76-25.79	

*The results are significant at $P<0.05$

Discussion

The distribution and characteristics nature of RVF have significantly changed in recent years. RVF has proven to be a major veterinary problem for dairy producers, wildlife managers, and veterinary diagnosticians due to the prevalence of sporadic cases and outbreaks in domestic and wild ruminants (Clark et al. 2018).

The prevalence and disease potential of RVF in Egyptian camels are both poorly understood. In clinical circumstances, camels are thought to be resistant to RVFV, and there are no symptoms of infection (Swanepoel and Coetzer 2004). Nonetheless, during the outbreak in Kenya from 2006 to 2007, clinical symptoms, fever, and abortion were observed in about 10% of pregnant she camels in free-ranging herds (Munyua et al. 2010). There has also been evidence of RVFV transmission among domestic camels (Britch et al. 2013; El-Harrak et al. 2011).

In this epidemiological survey, the overall seroprevalence rate for RVFV in camels in these four Egyptian governorates was 21.5%, which come in accordance with previous reported rate (21.11%) in camels in southern Egypt (Mahmoud and Ali 2021). This result considered as a high percentage of seropositivity, provide previous findings which indicating camels act as reservoir for maintenance of RVFV (Swai and Sindato 2015). Thus, a significant care should be implemented because of camels are used in Egypt for meat and milk production as well as in entertainment in the studied areas. There is some evidence to suggest that viremic, asymptomatic infected camels brought the virus to Egypt and likely also to the peninsula (Hoogstraal et al. 1979).

Table 2 Multivariable logistic regression analysis for potential risk factor associated with RVF in camels from Egypt

Factor	B	S.E.	OR	95% CI for OR		*P* value
				Lower	Upper	
Age						
>2-5	0.510	0.599	1.667	0.52	5.39	0.394
>5	1.501	0.598	4.49	1.39	14.49	0.012
Sex						
Female	1.217	0.412	3.38	1.51	7.58	0.003
Season						
June-August	1.789	0.614	5.98	1.79	19.93	0.004
December-February	0.475	0.639	1.61	0.46	5.63	0.457
March-May	0.033	0.639	1.03	0.29	3.62	0.959
Mosquitoes presence						
Yes	1.059	0.369	2.88	1.39	5.95	0.004
Mosquitoes control						
No	1.379	0.329	3.97	2.08	7.57	<0.0001
Body condition						
Emaciated	0.416	0.287	1.55	0.86	2.66	0.147

B Logistic regression coefficient, *SE* standard error, *OR* odds ratio, *CI* confidence interval

The prevalence in the current study differed from one governorate to another. The highest rates of infection were found in Kafr ElSheikh and Mersa Matrouh, which may be related to the raising of other animals including cattle, sheep, and goats alongside herds of camels (El Mamy et al. 2014). Also, the Nile Delta's unique microclimate, which is conducive to mosquito activity such as air temperature, humidity, the existence of permanent or intermittent sources of water and the vegetation index. Moreover, Mersa Matrouh received unregulated live animals and animal's products from some of neighbor countries especially Libya which have economic and political disorders. Besides, many of uncontrolled camels and small ruminants that were imported into the Aswan governorate originated from areas where the RVF virus is endemic, including the Sudan. These animals might be living, slaughtered and sold among other domesticated animals in the area, which could spread disease (Mroz et al. 2017).

The high prevalence rates for RVFV infection in camels were found in some countries in Africa such as Tanzania, Mauritania and Niger, it was 45% 38.5%, and 47.5%, respectively (El Mamy et al. 2014; Mariner et al. 1995; Swai and Sindato 2015). In addition, the seroprevalences were estimated to be 3.9% in Ethiopia, 22–36.4% in Kenya, over 30% in Egypt, 80% in Niger, 73.4% in Somalia, and 3.3% in Nigeria using the serum neutralization test (Davies et al. 1985, Hoogstraal, Meegan 1979, Mariner et al. 1995, Olaleye et al. 1996, Paweska et al. 2005). Using an inhibition ELISA test, the prevalence of RVFV in Camels were found 7.2% during 2007 in Kenya (Britch et al. 2013) and 45% in Mauritania (Jäckel et al. 2013).

The difference in seroprevelences of various studies in different countries might be attributed to animal age, season of sampling, sampling time (during outbreak or interepizootic), diagnostic method, mixing of animals under the same husbandry practices, administrative regions for camels and sustainability of ecological factors (Selim and Abdelhady 2020b; Selim et al. 2022a; Selim and Ali 2020; Selim et al. 2022b; Selim et al. 2022c; Selim et al. 2020a).

There was a substantial relationship between RVFV seropositivity and sex when considering sex as a risk factor, with seroprevalence being higher in females than in males. Contrary to our findings, other studies found higher seropositivity in females than males but without significant difference (Abdallah et al. 2015; Musa et al. 2020). In general, female animals stay in the herd for longer period than males. As a result, females are exposed to risk for RVF exposure for a longer time (Abdallah et al. 2015). This is consistent with the reports of high seropositivity with female sex as reported by El Mamy et al. (2011) in Mauritania and Sumaye et al. (2013) in Tanzania. This variation might result from different husbandry practices or from the different sample sizes for males and females.

Age-based data showed that camels older than 5 years had the highest prevalence. This age group has a high positive because it is the mature or adult age for camels when they graze freely in pastures or travel long distances where they may be exposed to mosquitoes and other infected hematophagous vectors. This is consistent with findings of Swai and Sindato (2015) and Di Nardo et al. (2014), they confirmed that elderly camels had higher infection risk for RFV. This might be explained by the fact that young

camels (those under two years old) are often kept indoors and are thoroughly cared for by their owners to avoid contracting infectious diseases, particularly those caused by insects and ticks (Said et al. 2022; Selim and Abdelhady 2020a; Selim et al. 2021b).

The findings also revealed that camels with small herd sizes had the highest rates of RVFV infection, whereas camels with medium-sized herd sizes had the lowest rates of infection. This is consistent with findings of Saeed and Aradaib (2017), where camels could be easily contract the RVFV infection more easily in small herd than in medium herd.

According to earlier studies, epidemics and outbreaks of the RVFV occurred over the summer and may have continued into the winter (Alhaji et al. 2018). High average temperatures and significant and unusual rainfall characterize the transition from summer to autumn, which generates ideal conditions for mosquito propagation and even the emergence of new species (Reiter 2001; Selim et al. 2021a; Selim and Radwan 2020). Also, the good weather and increasing precipitation, which produce mosquito breeding conditions for mosquito larvae, have been linked to this vector abundance.

The present result provided the activity of RVFV is sustained in mosquitoes in stagnant areas that do not totally dry out even during the months with less rainfall, allowing a low level of spreading to domestic animals, or that other risk factors other than precipitation are involved in the transmission of RVFV in Egypt (Linthicum et al. 2016; Zayed et al. 2015).

This study evaluated certain environmental risk variables that were significant for the prevalence of RVF in the study areas, such as the presence of mosquitos and/or the use of pesticides. The results were consistent with findings of Abdallah et al. (2015), where the high prevalence of RVF was significant associated with presence of mosquitoes. The principal mosquito species that could transmit the RVF virus are those belonging to the genera *Culex* (e.g., *Cx. Theileri* and *Cx. pipiens*) and *Aedes* (e.g., *Ae. detritus* and *Ae. caspius*). The primary vector for the transmission of the RVF and West Nile Fever (WNF) viruses in Egypt is the *Culex* mosquito, notably the *Cx. pipiens* (Baz et al. 2022b; Selim and Abdelhady 2020a; Selim and Radwan 2020b). This is related to its ecological adaptability, ability to endure in diverse bioclimatic regions, and aptitude to colonize various types of deposits and stagnant waters. The RVFV can spread and persist in all of North Africa, including Egypt, because of the ongoing unchecked movement of animals through smuggling and the existence of potential vectors. The prevalence and broad distribution of its most effective mosquito vector, *Cx. pipiens*, in Egypt make it suitable for supporting the circulation of RVFV (Meegan et al. 1980). As another factor contributing to the persistence of RVFV in endemic locations, transovarian transmission in arthropods is also important (Linthicum et al. 1988).

Conclusion

The study confirmed that RVFV circulate among camels in Egypt, as indicated by the presence of RVFV antibodies using cELISA. The seroprevalence of RVF in camels was significantly associated with sex, age, season and presence of mosquitoes. To better understand the epidemiology of RVF infection in Egypt, it is also necessary to conduct entomological surveillance of biting *Cx pipiens* implicated in the transmission of RVFV, as well as investigations of their ecology and epidemiology in the region. It is advised that routine monitoring and management of transboundary animal movements be implemented in the studied areas, together with increasing the public awareness about the disease's dangers, to lower the risk of the disease's spreading.

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Author contribution Conceptualization, methodology, formal analysis, investigation, resources, data curation, writing-original draft preparation, A.S., M.Z.S.A., K.A., M.S., M.K. and M.M.; writing-review and editing, A.S., M.Z.S.A., K.A., M.S., M.K. and M.M.; project administration, M.M.; funding acquisition, A.S., M.Z.S.A., K.A., M.S., M.K. and M.M. All authors have read and agreed to the published version of the manuscript.

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Data availability All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate The Benha University ethical committee for animal experiments approved all other procedures, including the handling and collection of blood samples. The animal owners gave their informed permission for the collecting of samples. The ARRIVE criteria were adhered to throughout the entire study process.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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