Survey of antibodies to Rift Valley fever virus and associated risk factors in one-humped camels (*Camelus dromedarius*) slaughtered in Maiduguri abattoir, Borno State, Nigeria

Hassan Ismail Musa¹ · Caleb Ayuba Kudi² · Muhammad Mamman Gashua¹ · Abubakar Sadiq Muhammad¹ · Abdulyeken Olawale Tijjani¹ · Adamu Saleh Saidu¹ · Sani Mohammed¹ · Saleh Mohammed Jajere¹ · Shuaibu Gidado Adamu¹

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Abstract

Rift Valley fever (RVF) is an emerging mosquito-borne zoonosis that threatens public health and animal agriculture in the endemic areas causing devastating epizootics characterized by abortion storms and high mortalities, especially in newborn animals. A cross-sectional study was conducted to determine the seroprevalence and investigate risk factors associated with exposure to the virus in camels slaughtered in Maiduguri abattoir, Borno State of Nigeria. Camels presented for slaughtered were sampled and data on age, sex, source or origin, utility, presence of post-mortem lesions, body weights and body condition score were collected. Blood samples were collected and sera were harvested and stored at – 20 °C until tested. The sera were tested using a commercial ELISA kit based on the manufacturer’s instructions. The overall seroprevalence in the study was 20.7% (95% CI, 13.6–30.0). The analysis showed no significant differences between the presence of antibodies and variables that included the sex of camels ($\chi^2 = 0.015, df = 1, p = 0.904$) and the presence of post-mortem lesion on the carcass ($\chi^2 = 0.009, df = 1, p = 0.925$). There were significant differences between presence of antibodies and three variables that included the age ($\chi^2 = 4.89, df = 1, p = 0.027$), the source ($\chi^2 = 7.077, df = 2, p = 0.029$) and the main utility ($\chi^2 = 8.057, df = 3, p = 0.045$) of the camels. It was concluded that camels presented for slaughter at the Maiduguri abattoir have evidence of exposure to the RVF virus and maybe means of transmission of the virus. Regular monitoring and control of transboundary animal movements were recommended in the study area.

Keywords Seroprevalence · Antibodies · Rift Valley fever · One-humped camel · Abattoir

Introduction

Rift Valley fever (RVF) is an emerging mosquito-borne zoonotic disease that threatens public health and animal agriculture in Africa and the Middle East. The causative agent, the RVF virus, is listed as a select agent with significant potential for international spread and use in bioterrorism and has been responsible for widespread and devastating outbreaks of severe disease throughout Africa and the Arabian Peninsula (Bird et al., 2009; Mahmoud and Ali, 2021). The agent is transmitted primarily by mosquitoes but could also be transmitted by direct contact with infected tissues and fluids (Linthicum et al., 2016; Leta et al., 2018). Epizootics and epidemics are usually recorded during years of exceptionally high rainfall. To date, RVF has been reported in over 30 countries in Africa in areas ranging from the dry, low-rainfall climate of Egypt (Kenawy et al., 2018), to the wet forest areas of Uganda and the Central African Republic, to the dry savanna lands of East and southern Africa (Glancey et al., 2015; Anyamba et al., 2018). Some possible mechanisms for spread include migrating or windblown vectors, movements or importations of viraemic mammals, phoresy of RVFV-infected ticks on migratory birds or movement of infected humans or vectors by aircraft (Linthicum et al., 2016). The epidemiology of the disease is very
complex and involves multiple players that include mosquitoes, wild animals, domesticated livestock and humans (Nanyingi et al., 2015). Cattle, sheep, goats and camels are particularly susceptible to RVF and serve as amplifying hosts for the virus (Linthicum et al., 2016). Epidemics of the disease are usually linked to persistent heavy rainfalls that favour the multiplication of its mosquito vectors (Linthicum et al., 2016). During the inter-epidemic period, the virus is primarily maintained by circulating at low levels among wildlife, livestock and humans, transmitted by mosquito vectors (Kariuki Njenga and Bett, 2019).

The one-humped camel, Camelus dromedarius, is an important pack and ride, dairy and meat animal in the semi-arid region of northeastern Nigeria (Jaji et al., 2017). They are usually kept for transportation as a draft animal and as a source of hides, milk and meat, and for sport in some countries (Ishag and Ahmed, 2011). These activities bring them in close contact with humans thus increased the chances to share infections. Most of the camels found in Nigeria were thought to have originated from eastern and northern Africa (Jaji et al., 2017) traverse through many countries on their way to the northeastern region of Nigeria. This may contribute to the spread of the RVF virus. Previous reports have shown evidence of the presence of the RVF virus in camels in Nigeria (Ezeifeke et al., 1982; Olaleye et al., 1996a,b; Opayele et al., 2019; Musa et al., 2020). A recent survey of domestic animals in the southwestern part of Nigeria indicated that the virus is actively circulating among domestic livestock in that region (Opayele et al., 2019). Similarly, a study from Borno State by Bukbuk et al. (2014) showed that RVF has comparatively the highest seroprevalence among the viral hemorrhagic fevers affecting humans in the state. However, there is a scarcity of published information on the disease in animals in the study area. Therefore, this study was conducted to determine the seroprevalence and investigate factors associated with exposure to the virus in camels slaughtered in Maiduguri abattoir, Borno State of Nigeria.

Materials and methods

Study area

The study was conducted at the Maiduguri abattoir located near the main livestock market within Maiduguri Metropolis. Maiduguri is the capital of Borno State, northeastern Nigeria. Maiduguri is located on latitude 11.8311° north and longitude 13.1510° east of the equator. It is the largest city in the northeastern part of Nigeria and covers a landmass of 132 km². The city is situated at about 325 m above sea level. According to the National Population Commission, the population of the city is estimated at 731,700 in 2015 with a projected population density of 5543.2 inhabitants per square kilometre. The main livestock market supplies animals for slaughter in the abattoir and used to be the biggest livestock market in the northeastern part of the country. Presently, livestock trading especially that of cattle is suspended due to security reasons. The animals traded in the market are sourced from within the country and from Chad, Sudan, Cameroon and the Niger Republic. This makes it an important point for interaction between disease agents and their respective hosts.

Borno State is divided into two broad relief regions, namely the hilly mountainous which covers the south and southwestern parts of the state and the plains which dominate most of the central and northern parts of the state. The climate is semi-arid with about two climatic seasons that include the wet season which starts from April to October with an average daily temperature of about 38.8 °C and the dry season which starts from November to March with an average daily temperature of about 36.8 °C. The two main vegetation zones are the Sudan savannah in the south and Sahel savannah in the north where Maiduguri is located.

Study design

A cross-sectional study was designed to determine the prevalence and investigate the risk factors associated with the presence of antibodies to the RVF virus in camels slaughtered in Maiduguri abattoir. The study covered three sampling periods. The first period targeted when camel slaughter figure was at its highest around January-December, the second period when slaughter figure was moderate and the third period when the figure is at the lowest based on previous records from the abattoir. This was to enable us to obtain a fair representation of slaughter figures in the abattoir.

Sample size determination

The unit of analysis considered in the study was an individual animal. The sample size of the cross-sectional study was calculated using the software, Statulator® Statistical Program (Dhand and Khatkar, 2014). The earlier reported prevalence of 3.3% in camels (Olaleye et al. 1996a,b) was utilized as the expected proportion. The statistical power of 80% was assumed and alpha (α) level; precision or margin of error was considered at 0.05. Based on the above assumptions, a minimum sample size of 45 camels was therefore required.

Alternatively, the same sample size was obtained using the formula for cross-sectional study (Thrusfield, 2007):

\[ n = \frac{z^2 \cdot p(1-p)}{d^2} \]
The sample size was adjusted to 92 to increase the precision of the estimate of the prevalence rate.

\[
\begin{align*}
\text{n} &= \frac{(1.96)^2 \times 0.03 \times (1 - 0.03)}{(0.05)^2} = 45
\end{align*}
\]

Therefore, a minimum of 45 serum samples was required for the study. However, the sample size was adjusted to 92 to increase the precision of the estimate of the prevalence rate.

### Inclusion criteria

Camels of all ages, both sexes, that were healthy, whose demographic information is known and is presented for slaughter at the Maiduguri abattoir, are eligible for inclusion into the study.

### Sample collection and handling

The camels used in this study were sampled using convenience sampling. Sterile sample bottles were used for the collection of the blood samples after which the samples were handled according to standard procedure (Olaleye et al. 1996a,b). The samples were allowed to clot at room temperature and transported to the laboratory in cold boxes with ice packs. The sera were separated from the clotted blood by centrifugation at 1500 g for 10 min. The harvested sera were stored at −20 °C until tested.

### Collection of camel demographic information

Camels presented for slaughtered that met the inclusion criteria were selected and included in the study. For every selected camel, the age, sex, source or origin, utility, body weight, body condition score and presence of post-mortem lesion on the carcass were recorded. The age of camels in the study was determined using rostral dentition through the phenomenon of teeth eruption and wearing (Bello et al., 2013). To ease data analysis on age, the camels were categorized based on age. Camels that were 5 years and below were categorized as young while those above 5 years were categorized as adults. This is because some camels attend sexual maturity at about 5 years of age (Rahim, 1997; Marai et al., 2009). The sex was determined by visual observation of the external genitalia.

The source and utility of the camels were determined through inquiry and interactions with owners or sellers. The body weights were estimated using body measurements that included chest girth, height at withers and hump girth (Boujenane, 2019). The body condition score was graded on a scale of 1 to 5 with a score of 5 being camel with an excellent body condition while a score of 1 is recorded for the camel with the worst body condition. This is because the body condition score is the external observable indicator of health status of the animal (Rahim, 1997; Islam et al., 2019). For each of the selected camels, a detailed post-mortem examination was conducted on the carcass and the presence or absence of lesions was noted. The information collected above was cross-tabulated with the occurrence of antibodies for the determination of the associated risk factors.

### Serological test

The serological test used in the study was based on the principles described by (Paweska et al., 2005). The test was performed using an enzyme-linked immunosorbent assay (ELISA) kit for the detection of anti-RVFV-nucleoprotein antibodies by competitive ELISA from serum or plasma. The commercial kit used was the ID Screen® Rift Valley Fever Competition Multi-Species kit from Innovative Diagnostics, ID. Vet, Montpellier, France. According to the manufacturer, the sensitivity of the kit was 100% (95% CI: 91.24–100%) while the specificity was 100% (95% CI: 99.54–100%) based on the validation study conducted in the endemic areas (Comtet et al., 2010).

The test procedure was as described by the manufacturer and was as follows:

- A 50 μl of the Dilution Buffer 19 was added to each of the 96 wells of the ELISA plate. Then, 50 μl of the positive control was added to well A1 and B1. A 50 μl of the negative control was added to well C1 and D1. Thereafter, 50 μl of each test sample was added to the remaining wells.
- The plate was incubated at 37 °C for 1 h. After the incubation, the wells were emptied and washed three times with 300 μl of the wash solution avoiding drying of the wells between washings. An Anti-RVF-NP Conjugate 1X was prepared by diluting the anti-RVF-NP-PO conjugate 10 × to 1/10 the dilution buffer 19. Thereafter, 100 μl of conjugate 1 x was added to each of the wells.
- The plate was incubated for the second time at 21 °C for 30 min. After the second incubation, the wells were emptied and washed three times with 300 μl of the wash solution avoiding drying of the wells between washings. Thereafter, 100 μl of the substrate solution was added to each of the wells. The plate was incubated for the third time at 21 °C for 15 min in the dark. Lastly, 100 μl of the stop solution was added to each of the wells to stop the reaction. The optical densities of the test solutions were read and recorded at 450 nm using an ELISA reader.
The test was validated by calculating the mean value of the optical densities (OD\textsubscript{NC}) of the negative control and was found to be 1.2005 which is greater than 0.7. Similarly, the mean value of the optical densities of the positive control (OD\textsubscript{PC}) was found to be 0.08 which was less than 0.30.

**Interpretation of the result of the test**

For each of the test samples, the competition percentage (S/N %) was calculated as follows:

\[
S/N\% = \frac{\text{OpticalDensity of sample}}{\text{OpticalDensity of Negative Control}} \times 100
\]

Samples presenting percentage competition of less than or equal to 40% were considered positive while those with greater than 40% were considered negative.

**Data analysis**

The data generated from the study were managed and stored in a Microsoft Excel\textsuperscript{®} 2011 for Mac OS X (Microsoft Corporation) spreadsheet. The seroprevalence rates were calculated by dividing the number of seropositive samples by the total number of samples using EpiTools Epidemiological Calculators (Sergent, 2014). The differences between proportions of the categorical variables were tested using the chi-squared test. The estimates and their respective confidence intervals were calculated at a 95% level using the Wilson (score) method described in a standard text (Brown et al., 2001). The mean values of the continuous variables were compared using an independent \( t \)-test. All the statistical analyses were performed using SPSS (version 23.0; SPSS Inc., Chicago, IL, USA) at the significance level of \( \alpha = 0.05 \).

**Results**

**Descriptive statistics and characteristics of camels in the study**

Out of the total 92 camels sampled in the study, 57 (62.0%) were females while 35 (38.0%) were males. For the age, the mean estimated age was 6.74 years with a standard deviation of 2.89 years. For the age categories, 29 (31.5%) of the camels were categorized as young while 63 (68.5%) were adults. The mean of the estimated live body weights was 331.85 kg with a standard deviation of 105.21 kg. In terms of the sources/country of origin of the camels, 25 (27.5%) camels were from Chad, 28 (30.4%) from the Niger Republic and 39 (42.4%) were sourced within Nigeria. For the body condition score, the average score for the camels in the study was 3.31 with a standard deviation of 0.88. In terms of the presence of post-mortem lesions, 67 (72.8%) of the camels had visible post-mortem lesions on their carcasses while 25 (27.2%) did not have any. In terms of the main purpose of keeping/utility, 11 (12.0%) were kept for the draft, 35 (38.0%) for meat, 25 (27.2%) for the milk and 21 (22.8%) for transportation purposes. The distribution of all the sampled camels based on the serological test for antibodies to RVF virus showed that 19 (20.7%) were positive while 73 (79.3%) were negative.

**Seroprevalence of RVF in camels slaughtered in Maiduguri abattoir**

The prevalence of antibodies against the RVF virus in the study area based on the investigated risk factors is shown in Table 1 below. The overall prevalence of antibodies against RVFV in the study area was found to be 20.7% (95% confidence interval, 13.6–30.0).

| Variable           | Categories | Total examined | Number positive | Prev. (%) | 95% CI of prevalence | \( p \)-value |
|--------------------|------------|----------------|-----------------|-----------|----------------------|--------------|
| Sex                | Female     | 57             | 12              | 21.1      | 12.5–33.3            | 0.904        |
|                    | Male       | 35             | 7               | 20.0      | 10.0–35.9            |              |
| Age                | Young      | 29             | 2               | 6.9       | 2.9–22.0             | 0.027*       |
|                    | Adult      | 63             | 17              | 27.0      | 17.6–39.0            |              |
| Source/origin      | Chad       | 25             | 7               | 28.0      | 14.3–47.6            | 0.029*       |
|                    | Niger      | 28             | 9               | 32.1      | 17.9–50.7            |              |
|                    | Nigeria    | 39             | 3               | 7.7       | 2.7–20.3             |              |
| PM lesion present  | No         | 67             | 14              | 20.9      | 12.9–32.1            | 0.925        |
|                    | Yes        | 25             | 5               | 20.0      | 8.9–39.1             |              |
| Main usage         | Draft      | 11             | 5               | 45.5      | 21.3–72.0            | 0.045*       |
|                    | Meat       | 35             | 3               | 8.6       | 3.0–22.4             |              |
|                    | Milk       | 25             | 5               | 20.0      | 8.9–39.1             |              |
|                    | Transport  | 21             | 6               | 28.6      | 13.8–50.0            |              |

*Significantly different, CI, confidence interval; Prev, prevalence
The chi-squared test showed no significant difference based on sex ($\chi^2 = 0.015, \text{df} = 1, p = 0.904$) in terms of the presence of the antibodies to the RVF virus. The comparison based on age showed a significant difference between age and the presence of antibodies ($\chi^2 = 4.89, \text{df} = 1, p = 0.027$) in the camels. Similarly, the comparison based on sources/origin of camels showed a significant difference between source and ($\chi^2 = 7.077, \text{df} = 2, p = 0.029$) presence of antibodies. The test also showed no significant difference between the presence of antibodies and the occurrence of post-mortem lesions on carcasses ($\chi^2 = 0.009, \text{df} = 1, p = 0.925$). For the main purpose of keeping the camel or its utility, the test showed a significant difference between the presence of antibodies and the main utility/purpose of keeping the camel ($\chi^2 = 8.057, \text{df} = 3, p = 0.045$).

Descriptive statistics and results of $t$-test analysis for the body weights and body condition score of the camels with positive and negative serological tests are shown in Table 2 below. The analysis showed no significant difference ($p > 0.05$) between the mean of the body weights of camels that tested positive and those that were negative. Similarly, there was no significant difference ($p > 0.05$) between the mean of the body condition scores of camels that tested positive and the mean for those that were negative. A Pearson correlation analysis showed a weak non-significant positive correlation ($r = 0.153, 95\% \text{ CI} = 0.053–0.347, p = 0.144$) between body weights and body condition scores of the camels sampled in this study. This means there is no significant relationship between the body weights and the body condition scores of the camels sampled in the study.

**Discussion**

The 20.7% seroprevalence reported in this study is observed to be relatively higher than the 5.3% reported in livestock handlers (Opayele et al., 2018) and the 0.7% (Opayele et al., 2019) among livestock slaughtered in Bodija Municipal abattoir in Ibadan, southwestern Nigeria. The observed disparity may be explained by the differences in species of animal sampled and the area studied. This is because camels may be at a higher risk of exposure to the virus due to their attributes of being transboundary animals that traverse borders thereby exposing them to higher risk (Faye, 2019). Moreover, the location of Maiduguri in northeastern Nigeria is considered to be closer to the hotspots of the disease in the north and east Africa (Arsevska et al., 2016; Lindahl et al., 2017) compared to Ibadan, which is located in southwestern Nigeria which is further away and considered to be at a lower risk. Similarly, the overall seroprevalence reported in this study was observed to be relatively higher when compared to the previously reported prevalence of 3.3% by (Olaleye et al. 1996a,b) and the 3.13% by (Ezeifeka et al., 1982) in Kaduna and Sokoto states of Nigeria. The observed disparity may be due to changing dynamics in the population of camels and assay methodology used in the detection of antibodies against RVFV. This is because the earlier studies (Ezeifeka et al., 1982; Olaleye et al. 1996a,b) used the haemagglutination-inhibition test while the present study used ELISA which is considered to be more sensitive.

The overall seroprevalence of 20.7% reported in this study was observed to be almost similar to the 19.9% reported in a recent study from the two northwestern states of Jigawa and Katsina in Nigeria (Musa et al., 2020). This may be because the two states share similar characteristics with Borno State since all the three states share international borders with other countries closer to the hotspots of the disease. In addition, the three states have similar climatic conditions thus exposed to similar risks of the disease (Caminade et al., 2011). Similarly, the seroprevalence reported in this study (20.7%) is observed to be almost similar to a recent report from southern Egypt that reported 20.65% in camels (Mahmoud and Ali, 2021) in which the authors opined that this high prevalence of the disease was a principal limiting factor for the livestock industry. The seroprevalence rate reported in camels in this study was also observed to be higher than the 14.1% reported in humans in Borno by Bukbuk et al. (2014) and this may suggest that camels have higher exposure rates when compared to the rates in humans.

Another reason may be attributed to the period of sample collection which may coincide with the increase in vector population in the study area. This is maybe a reflection of the seasonal trend of the disease. The overall seroprevalence reported in this study is, however, observed to be lower than the 34% reported in Tunisia (Selmi et al., 2020). This may be due to the fact Tunisia is located in the hotspots of the disease and thus has more favourable climatic conditions for the occurrence of the disease.

The analysis of the possible risk factors of the disease showed no statistically significant association between the

| Table 2  | Descriptive statistics and $t$-test analysis of camels with positive and negative antibodies against RVF virus |
|----------|--------------------------------------------------------------------------------------------------|
| Variable          | Range | Mean   | SD     | Positive ($n = 19$) | Negative ($n = 73$) | $p$-value |
| Body weight       | 350.0 | 331.8  | 105.2  | 292.1            | 342.2             | 0.064     |
| Body condition score | 3.0   | 3.32   | 0.88   | 3.3              | 3.3               | 0.773     |

*Significantly different, SD, standard deviation; SEM, standard error of the mean*
presence of antibodies to the virus and the sex of the camels. This is in contrast with the previous report by Muturi et al. (2021) that reported a significant association between infection and sex of camel in Kenya. This disparity may be due to differences in climatic zones and husbandry practices in the two study areas. It may also be as a result of the relatively small sample size in this study which might fail to detect the association. The study showed that age was a significant risk factor for the presence of antibodies and this agrees with previous reports from Kenya (Muturi et al., 2021) and Sudan (Abdallah et al., 2016). This may be because the older camels, having spent a long time in the environment, might have been exposed to greater risk when compared to young (Abdallah et al., 2016; Selmi et al., 2020). The study showed that the source or locality of the camel was a significant risk factor for exposure to the virus and this also agrees with previous reports from Kenya (Muturi et al., 2021) and Sudan (Abdallah et al., 2016). This may be explained by the fact that RVF is a vector-borne disease that occurs in specific ecologies, meaning that camels from different locations have varying exposure rates to different factors influencing the occurrence of the disease (Ikegami and Makino, 2011). The study showed no significant difference between the presence of antibodies and the occurrence of post-mortem lesions on carcasses. This is because camels are thought to be less likely to manifest the clinical form of the disease compared to other ruminants (Davies and Martin, 2006; Kwaśnik et al., 2021; Mahmoud and Ali, 2021). This may be the reason for the absence of lesions on carcasses of affected camels (Munyuia et al., 2010). This may constitute a serious risk since infected animals may not show clinical signs but may continue to serve as sources of infection to others. Finally, this study showed that the main utility of camel was a significant risk factor for the presence of antibodies. This is because camels are exposed to varying degrees of risk under different uses (El-Harrak et al., 2011; Napp et al., 2018).

The limitation of this is that the researchers relied on the retentive memories of the owners or sellers of the camels to collect data on the variables ‘source/origin of the camels’ and ‘utility of the camels’ with no other means to confirm the authenticity of the responses. This could be a potential source of recall bias.

It can be concluded from this study that camels presented for slaughter for human consumption at the Maiduguri abattoir have evidence of exposure to RVF virus and therefore constitute a risk to the abattoir personnel working, the general public and other livestock in the study area since they may serve as possible sources for transmission of the virus. The study revealed an overall high seroprevalence of 20.7% (95% confidence interval, 13.6–30.0) of the disease in camels in the study area. It can also be concluded that the sex of camels and presence of post-mortem lesions on their carcasses were not significant risk factors for exposure to RVF virus while the age, source or origin of the camel and the main usage/utility of the camels were significant risk factors for exposure to the virus.

To reduce the risk of occurrence of the disease, it is recommended that regular monitoring and control of trans-boundary animal movements be instituted in the study area in addition to carrying out public enlightenment on the dangers of the disease. Also, control of mosquito vectors and vaccination of camels during the high-risk period can go a long way in reducing the risk of transmission of the virus. Further research is needed in the study area to identify the possible sources of infection with the virus in the study area. There is also a need to conduct further research to determine the role of camels in the transmission of the RVF virus to other livestock species and humans in the study area. This information will be useful for designing more effective surveillance systems and intervention measures against the disease.

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Author contribution Conceptualization: Hassan Ismail Musa, Muhammad Mamman Gashua; methodology: Hassan Ismail Musa, Mohammed Sani, Muhammad Mamman Gashua; formal analysis and investigation: Hassan Ismail Musa, Abu Bakar Sadiq Muhammad; writing—original draft preparation: Hassan Ismail Musa, Muhammad Mamman Gashua; writing—review and editing: Hassan Ismail Musa, Abdulyeken Olawale Tijjani, Saleh Mohammed Tajere, Shuaibu Gidado Adamu, Adamu Saleh Saidu; supervision: Caleb Ayuba Kudi.

Data availability The datasets used and analysed in this study are available from the corresponding author on request.

Declarations

Ethics approval This is an observational study. The University of Maiduguri, Faculty of Veterinary Medicine Animal Use and Ethics Committee has confirmed that no ethical approval is required.

Conflict of interest The authors declare no competing interests.

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