Seroepidemiological Study of Interepidemic Rift Valley Fever Virus Infection among Persons with Intense Ruminant Exposure in Madagascar and Kenya

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Abstract. In this cross-sectional seroepidemiological study we sought to examine the evidence for circulation of Rift Valley fever virus (RVFV) among herders in Madagascar and Kenya. From July 2010 to June 2012, we enrolled 459 herders and 98 controls (without ruminant exposures) and studied their sera (immunoglobulin G [IgG] and IgM through enzyme-linked immunosorbent assay [ELISA] and plaque reduction neutralization test [PRNT] assays) for evidence of previous RVFV infection. Overall, 59 (12.9%) of 459 herders and 7 (7.1%) of the 98 controls were positive by the IgG ELISA assay. Of the 59 ELISA-positive herders, 25 (38.9%) were confirmed by the PRNT assay (21 from eastern Kenya). Two of the 21 PRNT-positive study subjects also had elevated IgM antibodies against RVFV suggesting recent infection. Multivariate modeling in this study revealed that being seminomadic (odds ratio [OR] = 6.4, 95% confidence interval [CI] = 2.1–15.4) was most strongly associated with antibodies against RVFV. Although we cannot know when these infections occurred, it seems likely that some interepidemic RVFV infections are occurring among herders. As there are disincentives regarding reporting RVFV outbreaks in livestock or wildlife, it may be prudent to conduct periodic, limited, active seroepidemiological surveillance for RVFV infections in herders, especially in eastern Kenya.

INTRODUCTION

Since its first discovery in 1931,1,2 Rift Valley fever virus (RVFV) has been detected in various sub-Saharan countries, as well as Egypt, Saudi Arabia, and Yemen, causing numerous outbreaks among both animals and humans.3–6 Kenya’s most recent Rift Valley Fever (RVF) outbreak of 2006–2007 spread to multiple provinces and districts and resulted in nearly 400 cases of severe illness with at least 118 human deaths.5,7 Epidemiological data collected from some of the patients demonstrated that two-thirds were exposed to a recently ill animal before infection.8 In addition, data suggested that other risk factors, including drinking raw milk, owning an ill animal, working as a herdsman, and slaughtering an animal, were also associated with RVFV infection.5,8

From January to May 2008 and from November 2008 to March 2009, a RVFV strain, similar to that identified in the 2006–2007 outbreaks in Kenya, was identified as the causative agent in human and animal outbreaks across Madagascar, which resulted in a total of 26 human deaths.9 However, this was not the first epizootic to occur in Madagascar, as outbreaks were also reported in the east coast in 1990 and 1991, which resulted in increased abortion rates among pregnant cattle by 17% and 15%, respectively.10,11 Following these outbreaks, it has been strongly suggested that enhanced surveillance should be implemented to more effectively predict and respond to future outbreaks.9 Though positive gains have been made to monitor RVFV in these countries, little is known regarding the maintenance of the virus during interepidemic periods.12–17 In an effort to better understand the ecology of human RVFV infections, we conducted this cross-sectional, seroepidemiological study of persons with intense exposure to ruminants living in eastern Kenya, western Kenya, and Madagascar.

METHODS

Study settings and design. This study was approved by Western Institutional Review Board and institutional review boards from collaborating institutions at each of the study sites (eastern Kenya—KEMRI Non-SSC no. 291, western Kenya—KEMRI SC1701, and Madagascar). Study personnel from each study site used informed consent to enroll participants ≥18 years of age who had a history of contact with ruminants. In Madagascar, participants were enrolled from the districts of Antsirabe, Antsoihiby, Ihosy, Maniranzavo, Nosy Be, Toliara, Toliara II, and Tsirioanomandidy during the period January–March 2012 (Figure 1A). In eastern Kenya, participants were enrolled from the villages of Gababa, Haji Mohamed, Hathama Chari, and Masalani in the North Eastern Province (Figure 1B) during February 6–12, 2012. In western Kenya, participants were enrolled from the Western Province (Figure 1B) during July 2010 to June 2012.

Ruminant exposure was defined as having an average of one or more cumulative hours per week exposure to camels, cattle, goats, or sheep, either by contact through touching and/or coming within 1 m of such animals during the 12 months before enrollment. Participants enrolled as controls resided in the same areas, denied having such contact, and when possible, were loosely age, group, and gender matched to exposed participants based on an expected final distribution.
of exposed study participants. Exclusion criteria for both groups included individuals less than 18 years of age, having any reported immunosuppression, or having been identified as medically likely to have greater susceptibility to various infectious agents.

Sample collection. Upon enrollment, participants completed an enrollment questionnaire with questions about demographics, animal and environmental exposures, and relevant medical information. Participants then permitted a serum sample collection, which was preserved at −80°C. Aliquots of serum were later shipped on dry ice to the University of Florida Emerging Pathogens Institute where they were first screened for human anti-RVF immunoglobulin G (IgG) antibodies with an enzyme-linked immunosorbent assay (ELISA). ELISA-positive samples were then tested with a plaque reduction neutralization test (PRNT) for validation. Finally, PRNT-positive samples were tested for human anti-RVF IgM antibodies with ELISA to delineate acute infections.

IgG enzyme-linked immunosorbent assay. Sera received from Madagascar and Kenya were first heat inactivated for 30 minutes at 56°C and then screened for IgG antibodies using a commercial RVFV human IgG ELISA kit obtained from Biological Diagnostic Supplies Limited (Scotland, United Kingdom) according to the manufacturer’s instructions. In brief, plates were coated with a recombinant nucleocapsid RVFV antigen diluted 1:1,000 in sodium bicarbonate buffer (pH = 9.6), covered with plate seals, and incubated at 4°C overnight. Unbound antigen was removed by washing three times for 15 seconds each using phosphate-buffered saline with 0.05% Tween 20 (PBS-T). Plates were then blocked with 10% (w/v) skimmed milk powder (SM) in PBS at 37°C for 1 hour. Plates were washed with PBS-T, test sera added in duplicate at a dilution of 1:400 in PBS + 2% (w/v) SM, and incubated for 1 hour at 37°C. Plates were washed once more, and horseradish peroxidase (HRP)-conjugated antihuman IgG antibody, diluted 1:25,000 in PBS + 2% (w/v) SM, was added to each well and incubated for 1 hour at 37°C. After a final wash, chromogenic detection of HRP was performed by the addition of 0.1 mL of the peroxidase substrate 3,3′,5,5′-tetramethylbenzidine (TMB) (KPL, Inc., Gaithersburg, MD), at room temperature for 10 minutes and stopped by the addition of 0.1 mL 1 N sulfuric acid. Absorbance of each well at 450 nm (A450) was measured by a PowerWave HT microplate spectrophotometer (Biotek, Winooski, VT). Negative and positive control sera were included for each plate. Sera samples were considered positive if their optical density (OD) calculation was ≥ 0.29 (net OD serum/net mean OD positive control).

Plaque reduction neutralization test. All samples testing positive by the ELISA kit were further studied using PRNT adapted from methods previously described. RVFV MP-12 vaccine strain, propagated in Vero-CCL81 cells, was used in the PRNT assay. Sera were tested in duplicate using six 4-fold dilutions starting with 1:10 and ending at 1:10,240. A back titration of the diluted stock MP-12 virus was performed each time assays were run to ascertain the titer of virus stock used (typically, 30–60 plaque forming units/mL). A neutralization cutoff of 80% reduction, as determined by a corresponding back titration plate, was used to determine sera titration.

IgM enzyme-linked immunosorbent assay. To ascertain whether an individual had evidence of an acute infection, an indirect capture ELISA, adapted in-house following the principles of Pawska and others, was used. Because of having
a limited amount of test serum and reagents, IgM testing was performed only for individuals who tested positive with the PRNT assay. First, 96-well microtiter plates were coated with a goat antihuman IgM antibody (catalog no. 01-10-03; KPL, Inc.) at a dilution of 1:2,000 in sodium bicarbonate buffer (pH = 9.6), covered with plate seals and incubated at 4°C overnight. Unbound antibody was washed from the well with PBS-T, and plates were then blocked with PBS with 5% (w/v) SM at room temperature for 2 hours. Test sera, diluted 1:100 in PBS-T plus 5% (w/v) SM, were added to coated plates and allowed to incubate for 1 hour at 37°C. Gamma-irradiated RVFV antigen, obtained from BEI Resources (National Institute of Allergy and Infectious Diseases, National Institutes of Health, RVFV, ZH501, Gamma-irradiated, NR-37380), was diluted 1:1,000 in PBS-T with 5% (w/v) SM, added to the plates, and allowed to incubate for 1 hour at 37°C. Rabbit anti-RVFV polyclonal antibody, obtained from Integrated Biotherapeutics Inc. (catalog no. 04-0001; Gaithersburg, MD), was diluted 1:1,000 in PBS-T with 5% SM, added to the plates, and allowed to incubate for 1 hour at 37°C. Extra serum adsorbed HRP-conjugated goat anti-rabbit IgG antibody (catalog no. 074-15-061, KPL, Inc.) was diluted 1:2,000, added to each well, and incubated for 1 hour at 37°C. All wells were washed five times after each incubation step using PBS-T. Each plate contained a "no antigen" negative control well to adjust for background absorbance. Chromogenic detection of HRP and plate reading was performed as described above. An IgM-positive control sample was not available for this assay. Instead, serum samples collected from six individuals with no possible RVFV exposure were collected and included in the assay run. IgM positivity was defined as any sample with an average A450 OD greater than three times the standard deviation plus the average A450 OD of the six negative control sera.

Statistical analysis. Bivariate $\chi^2$ tests of independence or Fisher's exact test were used to examine the association of demographic variables where PRNT serological outcomes were available. ELISA IgG positivity was used as the outcome variable when PRNT serological outcomes were not available. Variables determined by bivariate analyses to be statistically associated with RVFV seropositivity ($P < 0.25$) were then entered into a multivariable unconditional logistic regression model. Backward elimination was performed and covariates with $P < 0.05$ were retained in the model. Individual predictors retained in the final logistic models were tested for collinearity using bivariate $\chi^2$ tests. Finally, Hosmer-Lemeshow $\chi^2$ statistics for goodness of fit were performed. All demographic statistics, bivariate testing, and logistic modeling were conducted using SAS version 9.3 (SAS Institute, Cary, NC).

RESULTS

Study population. In Madagascar, participants were enrolled from the north, central, and south regions of the country, representing each of the unique climatic regions (Figure 1). Madagascar is an island nation with a population of nearly 23 million, located approximately 250 miles off the eastern coast of the African continent, south of the Equator. It is bordered to the west by the Mozambique Channel and

### Table 1

Demographic characteristics of the study participants enrolled in 2012, Madagascar and Kenya

| Demographic characteristics | Exposed* | Nonexposed | Exposed* | Nonexposed |
|-----------------------------|----------|------------|----------|------------|
|                             | n (%)    | n (%)      | n (%)    | n (%)      |
| Total                       | 93 (100) | 34 (100)   | 230 (100)| 136 (100)  |
| Gender                      |          |            |          |            |
| Male                        | 82 (88)  | 11 (32)    | 76 (33)  | 61 (45)    |
| Female                      | 10 (11)  | 24 (71)    | 154 (67) | 75 (55)    |
| Age group (years)           |          |            |          |            |
| 18–28                       | 32 (34)  | 10 (29)    | 58 (25)  | 54 (40)    |
| 29–44                       | 37 (40)  | 11 (32)    | 100 (44) | 39 (29)    |
| 45–60                       | 18 (19)  | 10 (29)    | 55 (24)  | 25 (18)    |
| > 60                        | 5 (5)    | 4 (12)     | 17 (7)   | 18 (13)    |
| Madagascar (districts)      |          |            |          |            |
| Antsirabe                   | 8 (9)    | 2 (6)      |          |            |
| Antsiohiny                  | 5 (5)    | 3 (9)      |          |            |
| Ilhouy                      | 44 (47)  | 16 (47)    |          |            |
| Miandrivazo                 | 10 (11)  | 9 (26)     |          |            |
| Nosy Be                     | 4 (4)    | 1 (3)      |          |            |
| Toliara                     | 0 (0)    | 1 (3)      |          |            |
| Toliara II                  | 12 (13)  | 1 (3)      |          |            |
| Tsroanomandidy              | 9 (10)   | 2 (6)      |          |            |
| Eastern Kenya (village)     |          |            |          |            |
| Gababa                      |          | 34 (15)    |          |            |
| Haji Mohamed                |          | 150 (65)   |          |            |
| Hathama Chari               |          | 17 (7)     |          |            |
| Masalani                    |          | 29 (13)    |          |            |
| Western Kenya (tribe)       |          |            |          |            |
| Japadhola                   |          |            |          |            |
| Kikuyu                      |          |            |          |            |
| Luhya                       |          |            |          |            |
| Luo                         |          |            |          |            |
| Samia                       |          |            |          |            |
| Teso                        |          |            |          |            |

*Exposure was defined as close contact through touching and/or coming within 1 m of a ruminant animal during the 12 months before enrollment.
to the east by the Indian Ocean. Participants were also enrolled from areas in the eastern and western regions of Kenya, a country of over 44 million in population. In eastern Kenya, individuals were enrolled in Garissa County, which is bordered by Somalia to the east and where RVFV cases have been previously reported. The human populations in this area are seminomadic pastoralists, which depend on livestock herds for survival. In western Kenya, participants were enrolled in the formerly named Western Province, which is bordered by Lake Victoria to the south and Uganda to the west. This region of Kenya has a mixed crop-livestock farming system and a high human population density, with a heavy endemic and epidemic disease burden on both humans and animals. Western Kenya contains a range of ecological settings from the Lake Victoria system in the south to a semi-mountain system on the lower slopes of Mount Elgon in the north.

We enrolled 127 participants (93 exposed and 34 controls) from Madagascar, 230 participants (all exposed) from eastern Kenya, and 200 participants (136 exposed and 64 controls) from western Kenya (Table 1).

**ELISA and plaque reduction neutralization test.** Of the 127 samples collected from Madagascar and tested by the ELISA assay, eight (6.3%) screened positive for IgG antibodies, of which two were confirmed positive by the PRNT assay at a sera dilution of 1:160 and 1:640. Between the two confirmed IgG PRNT-positive samples, both were from individuals with exposure to ruminants (two of 93 exposed = 2.15% RVFV positive) (Table 2). One of the two IgG PRNT-positive samples, which had a titer of 1:640, also tested positive by ELISA for IgM antibodies. This sample was collected from a man of 58 years, with daily reported exposure to cattle, who lived in Tsiroanomandidy and had no travel history outside Madagascar. This individual also reported monthly handling livestock farm animals. Western Kenya contains a range of ecological settings from the livestock-farming and a high human population density, with a heavy endemic and epidemic disease burden on both humans and animals. Western Kenya yields the outcome of choice for examining risk factor associations. Data from eastern Kenya were robust enough for examining PRNT as an outcome. However, as both Madagascar and western Kenyan sample populations yielded few PRNT positives, instead of PRNT, we examined ELISA IgG seropositivity as a surrogate for PRNT positivity. Bivariate and multivariate modeling results are recorded in Tables 3 and 4. Important bivariate risk factors for RVFV seropositivity included being seminomadic, drinking water from a public well or borehole, sleeping under a mosquito net, being bitten by a mosquito in the past 12 months, and wearing protective clothing when working with animals in the past 12 months (Table 3). Only the model for eastern Kenya yielded statistically significant risk factor associations with RVFV seropositivity: being seminomadic (odds ratio [OR] = 6.4, 95% confidence interval [CI] = 2.1-15.56) and sleeping under a mosquito net (OR = 3.2, 95% CI = 1.1-9.6). No collinearity problems were detected between any of the variables. Hosmer–Lemeshow X² statistics for goodness of fit indicated that predictors sufficiently described the data.

**DISCUSSION**

RVFV infections are considered as a major threat to the agricultural economies of many of the world’s nations where competent mosquito vectors are endemic. Although previously contained in Africa and Middle East, experts have argued that considering modern transportation and trade routes, RVFV poses a large threat to the European Union.
TABLE 3
Unadjusted ORs for risk factors associated with evidence of previous RVFV infection based on ELISA IgG seropositivity (Madagascar and western Kenya)

| Risk factor                                      | Total N | No. (%) | Unadjusted OR (95% CI) | Adjusted OR (95% CI) | Total N | No. (%) | Unadjusted OR (95% CI) | Adjusted OR (95% CI) |
|--------------------------------------------------|---------|---------|------------------------|----------------------|---------|---------|------------------------|----------------------|
| Ruminant exposure*                               |         |         |                        |                      |         |         |                        |                      |
| Yes                                              | 93      | 6 (6.5) | 1.1 (0.21, 5.8)        | –                    | 136     | 10 (7.4) | 0.94 (0.31, 2.9)        | –                    |
| No                                               | 34      | 2 (5.9) | Ref.                   | –                    | 64      | 5 (7.8)  | Ref.                   | –                    |
| Age (years)                                      |         |         |                        |                      |         |         |                        |                      |
| 18–28                                            | 42      | 3 (7.1) | 1.2 (0.28, 5.4)        | –                    | 75      | 6 (8.0)  | 1.1 (0.38, 3.3)         | –                    |
| 29–44                                            | 48      | 0 (0)   | –                      | –                    | 61      | 3 (4.9)  | 0.55 (0.15, 2.0)        | –                    |
| 45–60                                            | 28      | 4 (14.3)| 4.0 (0.92, 17.0)       | –                    | 34      | 2 (5.9)  | 0.74 (0.16, 3.4)        | –                    |
| > 60                                             | 9       | 1 (11.1)| 2.0 (0.22, 18.2)       | –                    | 30      | 4 (13.3)| 2.2 (0.66, 7.5)         | –                    |
| Gender                                           |         |         |                        |                      |         |         |                        |                      |
| Female                                           | 34      | 2 (5.9) | 0.91 (0.17, 4.7)       | –                    | 114     | 7 (6.1)  | 0.64 (0.22, 1.8)        | –                    |
| Male                                             | 93      | 6 (6.5) | Ref.                   | –                    | 86      | 8 (9.3)  | Ref.                   | –                    |
| Seminomadic                                      |         |         |                        |                      |         |         |                        |                      |
| Yes                                              | 1       | 0 (0)   | –                      | –                    | N/A     | N/A     | N/A                    | N/A                  |
| No                                               | 125     | 8 (6.4) | –                      | –                    | N/A     | N/A     | N/A                    | N/A                  |
| Drinking water from public well/borehole          |         |         |                        |                      |         |         |                        |                      |
| Yes                                              | 58      | 3 (5.2) | 0.70 (0.16, 3.05)      | –                    | 83      | 6 (7.2)  | 0.94 (0.32, 2.7)        | –                    |
| No                                               | 69      | 5 (7.2) | Ref.                   | –                    | 117     | 9 (7.7)  | Ref.                   | –                    |
| Sleep under a mosquito net                       |         |         |                        |                      |         |         |                        |                      |
| Yes                                              | 96      | 5 (5.2) | 0.51 (0.12, 2.28)      | –                    | N/A     | N/A     | N/A                    | N/A                  |
| No                                               | 31      | 3 (9.7) | Ref.                   | –                    | N/A     | N/A     | N/A                    | N/A                  |
| Bitten by a mosquito in past 12 months            |         |         |                        |                      |         |         |                        |                      |
| Yes                                              | 123     | 7 (5.7) | 0.51 (0.12, 2.3)       | –                    | N/A     | N/A     | N/A                    | N/A                  |
| No                                               | 4       | 1 (25.0)| Ref.                   | –                    | N/A     | N/A     | N/A                    | N/A                  |
| Wear protective clothing when working with animals in past 12 months |         |         |                        |                      |         |         |                        |                      |
| Yes                                              | 3       | 0 (0)   | –                      | –                    | N/A     | N/A     | N/A                    | N/A                  |
| No                                               | 116     | 8 (6.9) | –                      | –                    | N/A     | N/A     | N/A                    | N/A                  |
| Cared for birthing animal in past 12 months       |         |         |                        |                      |         |         |                        |                      |
| Yes                                              | 2       | 0 (0)   | –                      | –                    | 19      | 0 (0)   | –                      | –                    |
| No                                               | 124     | 8 (6.9) | –                      | –                    | 181     | 15 (8.3)| –                      | –                    |
| Butchered animal in past 12 months               |         |         |                        |                      |         |         |                        |                      |
| Yes                                              | 67      | 5 (7.5) | 1.5 (0.55, 6.7)        | –                    | 34      | 3 (8.8)  | 1.2 (0.33, 4.7)         | –                    |
| No                                               | 60      | 3 (5.0) | Ref.                   | –                    | 166     | 12 (7.2)| Ref.                   | –                    |
| Reported fever in past 12 months                 |         |         |                        |                      |         |         |                        |                      |
| Yes                                              | 46      | 4 (8.7) | 1.8 (0.44, 7.7)        | –                    | 120     | 8 (6.7)  | 0.74 (0.26, 2.1)        | –                    |
| No                                               | 81      | 4 (4.9) | Ref.                   | –                    | 80      | 7 (8.8)  | Ref.                   | –                    |

CI = confidence interval; ELISA = enzyme-linked immunosorbent assay; IgG = immunoglobulin G; N/A = data were not available for this covariate; OR = odds ratio; RVFV = Rift Valley fever virus; Ref. = Reference.

*Exposure was defined as close contact through touching and/or coming within 1 m of a ruminant animal during the 12 months before enrollment.

and the United States.22,23 Realizing this threat, the U.S. government is investing considerable funding in understanding the ecology of RVFV and in developing better diagnostics and vaccines.24 As part of this effort, we conducted this cross-sectional seroepidemiological study to assess human evidence of RVFV infection.

In general, our seroepidemiological study did not yield evidence for many unrecognized human RVFV infections in the three geographical areas we examined. However, because some of our seropositive study subjects were relatively young, our data suggest that indeed some human RVFV infections may be occurring during interepidemic periods. This would support the notion that passive surveillance for RVFV is not highly effective in detecting new epidemic threats. Our findings are also remarkable in that seropositivity was confirmed only among those with exposures to ruminants, and the risk factors of being seminomadic are consistent with our understanding of the ecology of RVFV infections.

The use of bed netting to reduce exposure to mosquitoes also had a positive association with RVFV seropositivity, which may seem unexpected; however, it may be explained by confounders that were not assessed by our survey. Possible confounders include differences in vector species and behavior, particularly as it relates to host seeking and feeding. For example, the protective effect of bed netting would be markedly reduced if RVFV mosquito vectors in a given area were predominantly daytime biters or displayed exophilic behavior. In addition, it is possible that individuals who reported using bed netting were also more likely to be in areas with higher densities of mosquitoes, resulting in an overall increased risk of exposure. This finding underscores the need to better understand vector ecology as it applies to RVFV transmission in interepidemic areas, however, it is to be noted that bed nets remain an effective method for the prevention of vector-borne diseases of public health importance such as malaria.

Our study had several limitations. Its cross-sectional nature makes it difficult for us to understand when the RVFV infections may have occurred among study subjects. Also, while we can infer some exposure information by the age of the seropositive subject, the precision of this association is poor. Prospective studies are a much better approach to estimating risk of human infection over time. The study is also limited in that we used slightly different approaches in sampling (sample population from eastern Kenya were all exposed) and in questioning the study subjects (instruments differed slightly between sites).

Considering our three sampling areas in this work and another similar study we conducted in Saudi Arabia,25 our
findings suggest that we are not missing large outbreaks of RVFV infections in the current surveillance and reporting systems. However, it does seem likely that small outbreaks, which affect both animals and man, may be missed or unreported. While one might argue that animals are the most sensitive sentinels for RVFV outbreaks, there are considerable disincentives for reporting RVFV outbreaks. Hence, conducting limited active surveillance for RVFV in man may be an important supplement to the surveillance that is conducted among domestic animals and wildlife. In particular, eastern Kenya would seem a good site to conduct such active surveillance for RVFV in herders. For instance, periodically screening subsets of herders for serological evidence of RVFV infection at various primary care clinics and hospitals in this region would seem a prudent and likely inexpensive additional early warning measure.

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### Table 4

Unadjusted and adjusted ORs for risk factors associated with evidence of previous RVFV infection by elevated PRNT assay (eastern Kenya)

| Risk factor                                      | Eastern Kenya | Total N | No. (%) | Unadjusted OR (95% CI) | Adjusted OR (95% CI) |
|--------------------------------------------------|---------------|---------|---------|------------------------|----------------------|
| Ruminant exposure*                               |               |         |         |                        |                      |
| Yes                                              | 0             | 0 (0)   | –       | –                      | –                    |
| No                                               | 230           | 21 (9.1)| –       | –                      | –                    |
| Age (years)                                      |               |         |         |                        |                      |
| 18–28                                            | 53            | 4 (7.5) | 0.95 (0.40, 2.2) | –                      |                      |
| 29–44                                            | 103           | 10 (9.7)| 0.75 (0.36, 1.6) | –                      |                      |
| 45–60                                            | 55            | 5 (9.1) | 1.8 (0.81, 3.8) | 0.32 (0.04, 2.5) | –                    |
| > 60                                             | 17            | 1 (5.9) | –       | –                      | –                    |
| Gender                                           |               |         |         |                        |                      |
| Female                                           | 154           | 15 (9.7)| 1.3 (0.5, 3.4) | –                      | –                    |
| Male                                             | 76            | 6 (7.9) | Ref.    | –                      | –                    |
| Seminomadic                                      |               |         |         |                        |                      |
| Yes                                              | 77            | 15 (19.5)| 5.9 (2.2, 16.0) | 6.4 (2.1, 15.4) | –                    |
| No                                               | 153           | 6 (3.9) | Ref.    | –                      | –                    |
| Drinking water from public well/borehole          |               |         |         |                        |                      |
| Yes                                              | 65            | 11 (16.9)| 3.2 (1.3, 7.9) | –                      | –                    |
| No                                               | 165           | 10 (6.0)| Ref.    | –                      | –                    |
| Sleep under a mosquito net                       |               |         |         |                        |                      |
| Yes                                              | 27            | 6 (22.2)| 3.6 (1.3, 10.2) | 3.2 (1.1, 9.6) | –                    |
| No                                               | 203           | 15 (7.4)| Ref.    | –                      | –                    |
| Bitten by a mosquito in past 12 months            |               |         |         |                        |                      |
| Yes                                              | 116           | 15 (12.9)| 2.7 (1.0, 7.2) | –                      | –                    |
| No                                               | 114           | 6 (5.3) | Ref.    | –                      | –                    |
| Wear protective clothing when working with animals in past 12 months | | | | | |
| Yes                                              | 17            | 4 (23.5)| 3.5 (1.0, 11.9) | –                      | –                    |
| No                                               | 210           | 17 (8.1)| Ref.    | –                      | –                    |
| Cared for birthing animal in past 12 months       |               |         |         |                        |                      |
| Yes                                              | 224           | 20 (8.9)| 0.39 (0.04, 3.7) | –                      | –                    |
| No                                               | 5             | 1 (20.0)| Ref.    | –                      | –                    |
| Butchered animal in past 12 months               |               |         |         |                        |                      |
| Yes                                              | 212           | 20 (9.4)| 1.6 (0.2, 12.5) | –                      | –                    |
| No                                               | 16            | 1 (6.3) | Ref.    | –                      | –                    |
| Reported fever in past 12 months                 |               |         |         |                        |                      |
| Yes                                              | 186           | 14 (7.5)| 0.43 (0.16, 1.1) | –                      | –                    |
| No                                               | 44            | 7 (15.9)| Ref.    | –                      | –                    |

CI = confidence interval; PRNT = plaque reduction neutralization test; OR = odds ratio; RVFV = Rift Valley fever virus.

*Exposure was defined as close contact through touching and/or coming within 1 m of a ruminant animal during the 12 months before enrollment.
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