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To cite this article: Jonas J. Wensman, Johanna Lindahl, Nica Wachtmeister, Emeli Torsson, Paul Gwakisa (Professor), Christopher Kasanga & Gerald Misinzo (Professor) (2015) A study of Rift Valley fever virus in Morogoro and Arusha regions of Tanzania – serology and farmers' perceptions, *Infection Ecology & Epidemiology*, 5:1, 30025, DOI: [10.3402/iee.v5.30025](https://doi.org/10.3402/iee.v5.30025)

To link to this article: <https://doi.org/10.3402/iee.v5.30025>



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Published online: 18 Nov 2015.



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ORIGINAL RESEARCH ARTICLE

A study of Rift Valley fever virus in Morogoro and Arusha regions of Tanzania – serology and farmers' perceptions

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Introduction: Rift Valley fever (RVF) is a zoonosis primarily affecting ruminants, resulting in epidemic abortions, fever, nasal and ocular discharges, haemorrhagic diarrhoea, and a high mortality rate among young animals. Rift Valley fever virus (RVFV) is an arthropod-borne RNA virus occurring in epizootic periods associated with heavy rainfall. The last outbreak of RVF in Tanzania was in 2006–2007, resulting in severe economic losses and impaired food security due to greater number of deaths of livestock. The aim of this study was to investigate the presence of antibodies against RVFV in sheep and goats in two different regions of Tanzania during an inter-epidemic period (IEP). In addition, the perception of important diseases among livestock keepers was assessed.

Material and methods: A cross-sectional serological survey was conducted in three purposively selected districts in Arusha and Morogoro regions of Tanzania. Serum samples from 354 sheep and goats were analysed in a commercial RVFV competitive ELISA. At the sampling missions, a questionnaire was used to estimate the socio-economic impact of infectious diseases.

Results and discussion: In total, 8.2% of the analysed samples were seropositive to RVF, and most seropositive animals were younger than 7 years, indicating a continuous circulation of RVFV in the two regions. None of the livestock keepers mentioned RVF as an important livestock disease.

Conclusions: This study confirms that RVFV is circulating at low levels in small ruminants during IEPs. In spite of recurring RVF outbreaks in Tanzania, livestock keepers seem to have a low awareness of the disease, making them poorly prepared and thus more vulnerable to future RVF outbreaks.

Keywords: *Rift Valley fever; small ruminants; epidemiology; vector-borne disease; arbovirus; awareness*

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Received: 14 October 2015; Revised: 21 October 2015; Accepted: 25 October 2015; Published: 18 November 2015

Rift Valley fever (RVF) is a zoonosis primarily affecting ruminants, resulting in epidemic abortions, fever, nasal and ocular discharges, haemorrhagic diarrhoea, and a high mortality rate among young animals. In humans, RVF mostly results in acute mild fever with spontaneous recovery, but in rare cases patients develop severe and fatal complications (1). Rift Valley fever virus (RVFV) belongs to the *Bunyaviridae* family, genus *Phlebovirus* (2), and occurs in epizootic periods associated with heavy rainfall (3). It is an arthropod-borne

virus, and believed to be primarily transmitted by mosquitoes of the genus *Aedes*, but other genera of mosquitoes are also able to transmit RVFV during epizootic periods (reviewed in Ref. 4). In East Africa, outbreaks have mainly been associated with prolonged rains and floods and El Niño Southern Oscillation (ENSO) (5).

Dense vegetation, suitable temperature conditions, and the presence of ruminants make it favourable for mosquitoes to breed, replicate the virus, and pass it on to animals and humans in Tanzania (6). Pastures in the lowland areas

overlap with hatching grounds of mosquitoes, increasing the risks for disease transmission (7). The major part of Tanzania's livestock is located in the northern and central regions of the country (8). In the whole country, there are an estimated 17 million cattle, 11 million goats, and 3.6 million sheep (8). Since RVFV was first identified in Kenya in 1930 (9), outbreaks in Tanzania have been reported in 1930, 1947, 1957, 1960, 1963, 1968, 1977–1979, 1989, 1997–1998, and 2006–2007, generally occurring between the months of December and June (10). In total, 39.2% of Tanzania's districts have been experiencing outbreaks, and the virus has been spreading southward from the Ngorongoro district in the north to the Ulanga district in the south. The outbreaks have varied in size and the inter-epidemic average period has been 7.9 years (10); however, antibodies to RVFV have also been detected among young animals during inter-epidemic periods (IEPs) and also in regions with no history of RVF outbreaks (6, 11, 12).

The latest RVF outbreak was reported in Tanzania in 2006–2007. The outbreak was officially announced on 7 February 2007 and ended in July 2007 (7), at which point 45 of 120 districts (10 of 21 regions) in Tanzania had been affected, with many human cases, often fatal (1, 10). The economic loss due to the death of domestic ruminants during the outbreak in Tanzania in 2006–2007 was estimated to reach US\$6 million; and additionally caused decreased food security and malnutrition for many people (10).

The aim of this study was to investigate the presence of antibodies against RVF in sheep and goats during the late dry season in two regions of Tanzania and to evaluate whether the virus is actively circulating during IEPs. This is of interest because studies indicate that the virus can circulate among animals and humans without causing clinical signs (6, 12). In addition, this study aimed to assess if livestock keepers perceived RVF as an important disease, and if they vaccinated against it, and if not, which diseases they did consider important.

Materials and methods

Study design

A cross-sectional serological survey was conducted in three purposively selected districts in Tanzania – Ngorongoro in Arusha region, and Mvomero and Ulanga in Morogoro region, during September–November 2014. Farmers were interviewed using a questionnaire, and small ruminants (goats and sheep) were sampled.

Questionnaire administration

At the sampling missions, a questionnaire was used to estimate the socio-economic impact of infectious diseases. The questions were asked to each livestock keeper through a local interpreter and data were collected on animals kept, vaccinations administered, common diseases, clinical

signs, foetal malformations, livestock management, disease management, and the perceived socio-economic impact of disease.

Animals and sampling

Blood samples were collected from 242 goats and 236 sheep among 39 different herds in three different districts in Tanzania during three separate field trips (Table 1). The blood was collected from the animal's jugular vein using a syringe, and serum vacutainer tubes without additives. When possible, equal proportions of sheep and goats in each herd were sampled. If there were individuals younger than 1 year in the herd, a few young animals were purposively sampled to get as broad age variation as possible. During sampling, the age of the animal was recorded, and if the farmer was not aware of this, the dentition of the sheep and goats was used to estimate the age (13). Each field trip was performed on 3 consecutive days and in order to be able to sample from as many herds and as many animals as possible, four herds were sampled per day, with approximately 15 animals sampled in each herd, depending on the size of the herd.

During the days in the field, blood samples were stored in a styrofoam box with ice packs, maintaining a temperature around 5°C in the box. At the end of the day, most of the collected blood samples were centrifuged and serum was stored in cryovials. However, because of field conditions, some of the samples were not separated until 3 days after they were collected. During this time, they were stored in a 5°C refrigerator. After the separation, all cryovials were stored at –45°C until serum was used for detection of RVFV antibodies.

RVFV competitive ELISA

A subset of samples was selected for testing for antibodies against RVFV using a competitive ELISA (ID Screen Rift Valley Fever Competition Multi Species, ID-vet, Grables, France), which detects both IgG and IgM antibodies directed against the nucleoprotein of RVFV. This test has been shown to have a high sensitivity (91–100%) and a high specificity (100%) in both tests done by the manufacturer

Table 1. Blood samples were collected from 478 individuals among 39 different herds in 15 villages

District	Herds <i>n</i>	Sheep <i>n</i>	Goat <i>n</i>	≥7 years <i>n</i>	Male <i>n</i>	Female <i>n</i>
Mvomero	12	83	94	27	41	135
Ngorongoro	14	66	87	3	51	101
Ulanga	13	87	61	1	33	115
Total	39	236	242	31	125	351

This table describes the distribution of species, sex, and also how many of the sampled individuals that were equal or older than 7 years. *n* = number of individuals.

and an independent ring trial (14). Samples were selected to get an equal proportion of goat and sheep from each herd. At first, 50% of the samples from each herd were analysed. After the first analysis, the remaining samples from the herds with a positive or doubtful result were also analysed. In total, 354 samples were analysed for the presence of antibodies against RVFV, corresponding to 74% of the total samples collected (Table 2). Of the 354 animals sampled, 330 individuals were younger than 7 years.

The competitive ELISA was performed according to the instructions of the manufacturer and all samples were run once. The absorbance was read at 450 nm. To control the validity of each plate, the mean value of the two negative controls (OD_{NC}) was calculated and the plate was considered valid when $OD_{NC} > 0.7$. For a valid plate, the mean value of the two positive controls divided by OD_{NC} should be < 0.3 . For each sample, the competition percentage was calculated by dividing (OD_{sample}/OD_{NC}) $\times 100$. If the value was equal or less than 40%, the sample was considered positive. A value greater than 50% was a negative result and the values in between 40 and 50% indicated a doubtful result.

Statistical analyses from the results of the serology were processed in Graphpad software in order to compare the seropositivity between sheep and goats. A confidence interval of 95% was used. When the proportion of seropositive animals was calculated, the doubtful individuals were considered as negative.

Results

Questionnaire

The livestock species in the three different areas were mostly sheep, goats, cattle, and chickens. The number of animals in each herd varied between the different study areas (Table 3).

All the villages visited also had dogs. Nineteen of the herds (48.7%) were not vaccinated for any disease during the last two years and none of the herds had been vaccinated against RVFV. In Mvomero district, the sheep and goats had been vaccinated against *peste des petits ruminants* (PPR). In herds in Ulanga district, most of the

cattle were vaccinated for lumpy skin disease in 2014 and some of the sheep and goats were vaccinated against PPR in 2013. The herds in the Ngorongoro Conservation Area were vaccinated against anthrax in 2014. In a few herds, only the sheep were vaccinated.

The livestock keepers mentioned several diseases as the most harmful diseases of their livestock, depending on herd and district. In Mvomero, PPR, brucellosis, and contagious caprine pleuropneumonia were considered as the main concerns. In Ngorongoro, the most feared disease was anthrax, whereas in Ulanga the farmers claimed parasites to be of major concern during the rainy season. The livestock keepers in Mvomero also mentioned several tick-borne diseases, for example anaplasmosis. Regarding which clinical signs caused major problems in the herd in Mvomero and Ngorongoro, the livestock keepers answered diarrhoea and pneumonia. In Ulanga, the livestock keepers answered diarrhoea but specified it to diarrhoea during the rainy season.

Several herds had contact with wildlife. In Mvomero, close to the Mikumi National Park, one herd had contact with bigger wild animals such as impala, wild dogs, and lion, whereas the 11 remaining herds had contact with dik dik, a small antelope. In Ulanga, the sheep and goats had contact with buffaloes and bush pigs and some of the cattle had contact with impalas. In Ngorongoro, the sheep and goats coexisted with a variety of wild animals within the Ngorongoro Conservation Area, for example, impala, buffalo, jackal, hyena, and zebra.

Four of 39 livestock keepers said that if a big amount of their animals get sick or die they would change boma, the fenced cattle paddock, and then burn the faeces and the dead animals to prevent contamination. All the livestock keepers explained that a massive loss of animals due to disease or other causes would affect their family, health, and economy very negatively.

Questions were also asked about the general health and mortality in the herds. Eighteen of 39 of the herds had during the last 2 years had an outbreak and 17 of the animal owners claimed it was due to PPR. The clinical signs observed during the outbreaks were diarrhoea, pneumonia, coughing, nasal discharge, high rate of abortions, and mortality among the young animals.

Table 2. Distribution of the 354 samples analysed by the RVFV-ELISA

District	Sheep <i>n</i>	Goat <i>n</i>	Total analysed <i>n</i>	≥ 7 years <i>n</i>
Mvomero	57 (69%)	67 (71%)	124 (70%)	20 (74%)
Ngorongoro	41 (62%)	64 (74%)	105 (69%)	3 (100%)
Ulanga	70 (80%)	55 (90%)	125 (84%)	1 (100%)
Total analysed	168 (71%)	186 (77%)	354 (74%)	24 (77%)

The percentage shows the percentage of the sampled individuals that were analysed. *n* = number of individuals.

Table 3. The approximate number (*n*) and mean of animals in each herd

Study areas District	Type of livestock			
	Sheep <i>n</i> (mean)	Goat <i>n</i> (mean)	Sheep + goat <i>n</i> (mean)	Cattle <i>n</i> (mean)
Mvomero			60–200 (130)	60–200 (130)
Ngorongoro	50–200 (150)	50–200 (125)		6–600 (303)
Ulanga			3–50 (26.5)	0–50 (25)

Serological results

In total, 39 herds in 15 different villages were sampled and 13 of these herds had at least one seropositive animal. In Ngorongoro, only 1 of 10 (10%) sampled herds was seropositive, whereas 6 out of 12 herds (50%) in Mvomero, and 6 of 13 herds (46%) in Ulanga were seropositive for RVF. The proportion of RVF seropositive animals varied within the different herds in Mvomero (0–55%), Ngorongoro (0–10%), and Mahenge (0–25%).

Of the sampled animals, 74% were analysed for RVF antibodies. In the first step, 272 samples were analysed (Table 4); and the remaining 82 individuals were analysed during the second step. Of the 354 analysed samples, 29 individuals were positive and 6 individuals were doubtful (Table 5).

The total proportion of seropositivity for all analysed samples was 8.2%. Mvomero and Ulanga districts had a similar proportion of seropositivity, 11.3 and 11.2%, respectively, which differed slightly between the first and second analysis steps. Ngorongoro had the lowest proportion of seropositivity of the three study areas; only 1 of 105 animals was seropositive. In total, the proportion of seropositivity in goats was slightly higher than in sheep, although in Ulanga the proportion of seropositivity in sheep was higher than in goats. Of the animals older or equal to 7 years, 25% were seropositive for RVFV antibodies, whereas 7% of the analysed animals younger than 7 years were seropositive. Five of the six seropositive animals older than 7 years were found in Mvomero district, where there also was the highest proportion of seropositive animals among the herds; one herd had 55% positive animals. There was no obvious difference in proportions of

seropositivity between samples where serum was separated within the same day of sampling or separated 3 days after sampling.

Discussion

In this study, the aim was to investigate the presence of antibodies against RVFV among sheep and goats and to evaluate whether the virus was active during the IEPs. The results indicate that 8.2% of the goats and sheep tested in this study have encountered RVFV sometime during their life. The latest outbreak in Tanzania with a subsequent vaccination campaign was in 2007, 7 years before this study (15). The seropositive animals younger than 7 years ($n=23$) did not live during the major outbreak and must have been exposed to virus after this outbreak. This strongly indicates that RVFV is circulating in low levels in the three districts during IEP. However, it is not possible to distinguish whether animals older than 7 years (21% of seropositive animals) are positive due to previous vaccination or if they were exposed during the outbreak, because of lack of tests that can differentiate infected from vaccinated animals (DIVA) (16).

Ngorongoro had only one seropositive animal, which was younger than 7 years, and thus exposed during the IEP. This low proportion of seropositive animals (1.0%) is unexpected because the district has been involved in all RVF outbreaks that have occurred in Tanzania (10); however, it was not assessed if the included farms had actually been experiencing outbreaks.

This study aimed at evaluating farmers' perceptions and to search for indications of seroconversions during the IEP, and the design does not allow for estimates of

Table 4. Results from the first-step ELISA, 272 animals were analysed, and 22 animals were seropositive

District	Sheep % (<i>n</i>)	Goat % (<i>n</i>)	Total % (<i>n</i>)	≥ 7 years % (<i>n</i>)
Mvomero	4.7 (2)	18.4 (9)	12.0 (11)	6.7 (1)
Ngorongoro	2.5 (1)	–	1.1 (1)	–
Ulanga	12.0 (6)	9.8 (4)	10.9 (10)	–
Total (%)	6.7 (9)	9.4 (13)	8.1 (22)	6.7 (1)

n = number of individuals.

Table 5. Results from ELISA showing the total seroprevalence and number of positive animals

District	Sheep % (<i>n</i>)	Goat % (<i>n</i>)	Total % (<i>n</i>)	≥ 7 years % (<i>n</i>)
Mvomero	3.5 (2)	18.0 (12)	11.3 (14)	30.0 (6)
Ngorongoro	2.4 (1)	–	1.0 (1)	–
Ulanga	14.3 (10)	7.3 (4)	11.2 (14)	–
Total (%)	7.7 (13)	8.6 (16)	8.2 (29)	25.0 (6)

In total 354 individuals were analysed. *n* = number of individuals.

seroprevalence due to the non-random sampling of animals and laboratory analyses. In total, only 74% of the animals sampled were analysed, because of restrictions in the number of ELISA kits, and the purposive screening of samples from herds where positive samples were detected means that the proportions of seropositivity reported here are likely to be higher than the true seroprevalence. However, the proportions of seropositivity in Mvomero and Ulanga districts correspond to other serological studies (11) and the proportion of seropositivity reported for all animals tested is similar to the proportion achieved before selecting animals from positive herds (8.1% compared to 8.2%), indicating that the bias is not so high. Samples sometimes had to be stored for days without separating the serum, but samples stored for 3 days still enabled detection of antibodies, thus indicating that, although it is possible that it could bias the results slightly, the suboptimal storage of samples is unlikely to make it impossible to detect seropositivity.

One herd in Mvomero district had the highest seropositivity (55%) among the herds. There were no clinical signs or results from the interview with the livestock keeper that could explain the high seropositivity from this particular herd, except that this herd had more contact with wild animals than the other herds around Mikumi National Park. The livestock keeper mentioned impala, wild dogs, and lions as some wildlife species in contact with the herd. Wildlife contact is perceived as a risk factor for RVF, and in many farms it was reported that the livestock were in contact with wildlife. On a couple of farms, sheep and goats were not reported to have direct contact with wild animals. These farms also had cattle, and cattle graze further away and may thus bring virus back after having wildlife contacts. Buffaloes and elephants in the northern part of Tanzania had been shown to have RVFV antibodies during the IEP 2002–2006 (17). Several studies on wildlife in Kenya also show the presence of antibodies (18–20), with lower proportions during the IEP and higher during epizootics (19).

Previous studies have shown the presence of antibodies to RVFV in different parts of Tanzania during the IEP. In 2004 seropositive humans were found in Tanga region (21), and a study in Ulanga in 2011 showed a seroprevalence of 13% in cattle, sheep, and goats (11), compared to 11.2% in our study from the same district, indicating a consistent circulation of RVFV. The seroprevalence in animals born after the outbreak in 2006–2007 was 5.5 and 22.7% in animals that had lived through the outbreak (11). Even IgM antibodies were found indicating a more recent infection in 2011, 4 years after the major outbreak (11). Antibodies have been detected in both humans and ruminants in areas with no experience of RVF epidemic (6, 12), and having cattle was associated with higher RVFV seroprevalence in humans (6). To conclude these different studies, findings of antibodies in animals and humans in

areas with no history of RVF outbreaks and in individuals younger than 7 years strongly indicate that RVFV is endemic and circulating in low levels in several regions in Tanzania.

The age estimates of the animals were mainly based on the reports of the farmer. When the farmer was uncertain about the age of the animal, teeth were used to estimate the age (13). This method is subjective and some animals may have been misclassified, which could influence the interpretation of which animals may have been exposed during the previous outbreak.

In this study, there was a higher proportion of seropositive goats (8.6%) than sheep (7.7%), although it was not statistically significant. This difference between sheep and goats would be good to follow up when a new screening is performed. Other studies have observed a higher seroprevalence of RVF in sheep (22, 23) or no significant difference between sheep and goats (11), similar to this study.

It is important to have an active surveillance regarding RVFV in endemic countries to prevent new outbreaks and to avoid the subsequent negative effect it has on the livestock, population, welfare, and economy. Even though the outbreaks reoccur in an irregular pattern it is worth keeping track of variations in ENSO that might lead to an increase in precipitation in a few months (24). When an increase in precipitation is expected, it is possible to vaccinate livestock in advance and inform the population on how to prevent transmission and infection.

In the interviews with livestock keepers, none of them mentioned RVF as being among the most feared diseases. Chengula (15) pointed out that a majority of the people interviewed in the regions Arusha, Morogoro, and Manyara did not know that RVF was an outbreak disease. There have also been indications that a big part of the population does not know that RVF is a zoonosis (21).

In the face of a possible new El Niño, it is important to remember that the farmers may need to be reminded and informed about RVF and how they can protect themselves and their livestock. The clinical symptoms of RVF in humans can resemble those of malaria and other febrile diseases. In the beginning of an outbreak it might therefore be easy to miss the diagnosis (25). If a malaria test is negative but the patient still has fever, it is worth considering RVF as a differential diagnosis, especially because both diseases increase when the mosquito population increases, often in association with heavy rainfall.

Of the herds visited, 44% reported having had an outbreak during the last 2 years and claimed it was due to PPR virus. Often diagnosis is based on clinical signs, and PPR is a differential diagnosis to RVF (26), which is good for local veterinarians to keep in mind as it can function as a warning signal for a new RVF outbreak.

In conclusion, this study shows the presence of animals seropositive to RVF 7 years after an outbreak, indicating that the virus may still be circulating in low numbers,

awaiting the next outbreak. Livestock keepers are not vaccinating against the disease, and not considering it a priority, which may mean that the farmers in rural Tanzania are poorly prepared for when it next happens.

Acknowledgements

We thank Dr. Sayalel Kuya (Veterinarian, Ngorongoro Conservation Area Authority), Dr. Petro J. Lema (District Veterinary Officer, Mvomero), and Dr. Emmanuel Lyimo (District Veterinary Officer, Ulanga) for facilitating field sampling, and Ida Herbe, Lovisa Levin, and Mariamu Makange for technical assistance. We also thank all livestock keepers for consenting to use their animals for this study.

Conflict of interest and funding

The authors declare no conflict of interest. This study was funded by the Swedish Research Council SRC 348-2013-6402. This study was part of an MSc thesis undertaken as a Minor Field Study (MFS) supported by the Swedish International Development Cooperation Agency (SIDA). Additional support was achieved from the Faculty of Veterinary Medicine and Animal Science of the Swedish University of Agricultural Sciences as a scholarship to NW.

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