Neutralizing antibodies against Rift Valley fever virus in wild antelope in far northern KwaZulu-Natal, South Africa, indicate recent virus circulation

Short title: Rift Valley fever virus circulation in wild antelope

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Summary

Rift Valley fever (RVF) is a zoonotic viral disease of domestic ruminants in Africa and the Arabian Peninsula caused by a mosquito-borne *Phlebovirus*. Outbreaks in livestock and humans occur after heavy rains favour breeding of vectors, and the virus is thought to survive dry seasons in the eggs of floodwater-breeding aedine mosquitoes. We recently found high seroconversion rates to RVF virus (RVFV) in cattle and goats, in the absence of outbreaks, in far northern KwaZulu-Natal (KZN), South Africa. Here we report the prevalence of, and factors associated with, neutralizing antibodies to RVFV in 326 sera collected opportunistically from nyala (*Tragelaphus angasii*) and impala (*Aepyceros melampus*) culled during 2016-18 in two nature reserves in the same area. The overall seroprevalence of RVFV, determined using the serum neutralization test, was 35.0% (114/326; 95%CI: 29.8-40.4%) and tended to be higher in Ndumo Game Reserve (11/20; 55.0%; 95%CI: 31.5-76.9%) than in Tembe Elephant Park (103/306; 33.6%; 95%CI: 28.4-39.3%) (P=0.087). The presence of antibodies in juveniles (6/21; 28.6%; 95%CI: 11.3-52.2%) and sub-adults (13/65; 20.0%; 95%CI: 11.1-37.8%) confirmed that infections had occurred at least until 2016, well after the 2008-2011 RVF outbreaks in South Africa. Odds of seropositivity was higher in adults than in sub-adults (OR=3.98; 95%CI: 1.83-8.67; P=0.001), in males than in females (OR=2.66; 95%CI: 1.51-4.68; P=0.001), and in animals collected ≤2 km from a swamp or floodplain compared to those collected further away (OR=3.30; 95%CI: 1.70-6.38; P<0.001). Under similar ecological conditions, domestic and wild ruminants may play a similar role in maintenance of RVFV circulation and either or both may serve as the mammalian host in a vector-host reservoir system. The study confirms the recent circulation of RVFV in the tropical coastal plain of northern KZN, providing the basis for investigation of factors affecting virus circulation and the role of wildlife in RVF epidemiology.

Keywords: Rift Valley fever virus; seroprevalence; antelope; zoonosis; wildlife.

Introduction

Rift Valley fever (RVF) is a zoonotic mosquito-borne disease of ruminants in Africa and the Arabian Peninsula caused by RVF virus (RVFV) within the genus *Phlebovirus,* family *Phenuiviridae* (Swanepoel & Coetzer, 2004; Paweska, 2015; Adams et al., 2017). Outbreaks of RVF are recognized by abortion storms in domestic ruminants and deaths of young animals (Swanepoel & Coetzer, 2004). Humans become infected by contact with tissues and body fluids of infected livestock, or less frequently from mosquito bites, and usually experience benign febrile illness, but may develop fatal haemorrhagic fever, encephalitis, or ocular sequelae (Wilson, 1994). The virus was discovered in Kenya in 1930 (Daubney, Hudson, & Garnham, 1931), and the disease was first recognized in South Africa in a major epidemic in 1950-51 (Gear, De Meillon, Measroch, & Davis, 1951), with further large-scale outbreaks occurring in 1974-76 (Barnard, 1977) and 2008-11 (Metras et al., 2012). Large outbreaks usually occur after exceptionally heavy rains that favour breeding of the mosquito vectors; in southern Africa, such circumstances tend to follow La Niña weather events (Anyamba, Linthicum, & Tucker, 2001).

The virus is believed to survive inter-epidemic periods through transovarial transmission in floodwater-breeding *Aedes* spp. mosquitoes (Linthicum, Davies, Kairo, & Bailey, 1985). Their eggs need to undergo a degree of desiccation followed by re-submergence in rainwater before hatching and producing infected adult mosquitoes. The virus is then transmitted to susceptible animals that in turn serve as a source of virus for competent mosquito vectors when taking viraemic blood meals (Swanepoel & Coetzer, 2004). When heavy rains result in a population explosion of mosquitoes, larger numbers of animals become infected, and *Culex* spp. mosquitoes that breed in more permanent water bodies then serve as epidemic vectors to intensify the outbreaks (Linthicum, Davies, Bailey, & Kairo, 1983).

During the first recognized outbreak in South Africa in 1951, abortions were observed to occur in antelope, including springbok (*Antidorcas marsupialis*) and blesbok (*Damaliscus dorcas dorcas*), although RVFV was not proven to be the cause (Alexander, 1951; Gear et al., 1951). In 1999 abortions in a waterbuck (*Kobus ellipsiprymnus*) and six African buffaloes (*Syncerus caffer*) were confirmed to be caused by RVFV (ProMED-mail, 1999). Unspecified clinical disease, including death, due to RVFV was reported in African buffalo, springbok, kudu (*Tragelaphus strepsiceros*), nyala (*T. angasii*), sable (*Hippotragus niger*), roan (*H. equinus*), gemsbok (*Oryx gazella*), blesbok, bontebok (*D. dorcas phillipsi*) and waterbuck during the 2010-2011 RVF outbreaks in the interior of South Africa, although in extremely low numbers compared to the large numbers of domestic ruminants affected (DAFF, 2011; Pienaar & Thompson, 2013).

Antibodies to RVFV have been detected in multiple wildlife species in Africa, including African buffalo, black rhino (*Diceros bicornis*), African elephant (*Loxodonta africana*) and several antelope (Bovidae) species (Bird et al., 2008; Evans et al., 2008; Beechler et al., 2015; Jori et al., 2015; Dondona et al., 2016). A study conducted in wildlife reserves in Kenya tested sera for RVFV antibodies from 16 species of wildlife; those with the highest seroprevalences included African buffalo (37/237; 16%), black rhino (14/43; 23%), Thomson's gazelle (*Eudorcas thomsonii*) (7/8; 87%), kudu (5/10; 50%), impala (*Aepyceros melampus*) (5/8; 62%) and waterbuck (2/10; 20%) (Evans et al., 2008). Although these sera were collected during an inter-epidemic period, many of the animals may have been infected during the previous epidemic; some evidence, however, of inter-epidemic circulation in wildlife was found.

A study using samples collected in 2003-2004 in South Africa found a prevalence of antibodies to RVFV in African buffalo in the Kruger National Park (KNP) of 6.5% and 4.5% in Hluhluwe-iMfolozi Park, KwaZulu-Natal, using a RVFV IgG ELISA (Fagbo, Coetzer, &

Venter, 2014). In the KNP a low rate of seroconversion was reported among buffaloes (9/126; 7%) over a six-year period (2000-2006) using a hemagglutination-inhibition titration assay (LaBeaud, Cross, Getz, Glinka, & King, 2011). Also in the KNP, 5/227 seronegative female buffaloes seroconverted to RVFV over a 5-year period (2008-2012), based on a serum neutralization test (Beechler et al., 2015), confirming a very low level of circulation in the absence of observed outbreaks.

It has been suggested that wildlife may serve as RVFV maintenance hosts during inter-epidemic periods, since areas rich in water sources and intermittent wetlands, along with Bovidae species, are positively associated with RVFV outbreaks (Walsh, de Smalen, & Mor, 2017). The potential role of wildlife in the epidemiology of RVF has been reviewed (Olive et al. 2012), and it was concluded that there is no definitive evidence for a wildlife maintenance host. However, due to the absence of a known carrier state, the maintenance "host" must by necessity be a host-vector system rather than a single species. In the KNP where very low-level seroconversion was found, it has been concluded in this study that a combination of mammalian hosts and vertical transmission by mosquitoes is necessary for RVFV persistence (Manore & Beechler, 2015).

We recently found high seroconversion rates to RVFV in cattle and goats in far northern KwaZulu-Natal (KZN) Province, South Africa, in the absence of reported outbreaks of disease (Van den Bergh, Venter, Swanepoel, & Thompson, 2019). The livestock tested in that study were in an area adjoining two nature reserves, although separated from wildlife by fences. In order to investigate the potential role of wildlife in RVFV circulation in the area, the objective of this study was to determine the seroprevalence and associated risk factors of RVFV in antelope in the Tembe Elephant Park (TEP) and the Ndumo Game Reserve (NGR), using sera from animals routinely culled over a two-year period.

Materials and methods

Ethical approval

The study was approved by the Animal Ethics Committee of the University of Pretoria (V013-16) and adhered to the specifications of the South African National Standard (SANS 10386-2008): "The Care and Use of Animals for Scientific Purposes". Support for the project was obtained from Ezemvelo KZN Wildlife, the KwaZulu-Natal conservation authority that manages the two reserves. The project was approved by the Department of Agriculture, Forestry and Fisheries, Republic of South Africa, and the KwaZulu-Natal Department of Agriculture and Rural Development (KZNDARD), which issued permits for the movement of animal samples from the foot-and-mouth disease-controlled area to the University of Pretoria.

Study area

The study was conducted in the ~30,000 ha TEP and the ~10,000 ha NGR on the Maputaland coastal plain of northern KwaZulu-Natal, which is bordered by the Lebombo Mountains to the west and the Indian Ocean to the east. The TEP and surrounding areas are covered by open woodlands with grasslands, palmveld and patches of sand forest (Moll & White, 1978; Matthews, Van Wyk, Van Rooyen, & Botha, 2001). The Muzi swamp with its reed beds crosses the eastern side of the reserve and constitutes the only permanent source of water in the park. The NGR, known for its diversity of bird life and large floodplain systems, is situated at the confluence of the Pongolo River and the Usuthu River, which forms the northern boundary of the reserve. The reserve is characterized by diverse habitats of riverine forest, floodplains, grasslands, reed beds, broad-leaved and acacia woodlands, and dense thornveld (Pooley, 1982; Calverley & Downs, 2014). Both reserves border Mozambique to the north, with the TEP border being fenced across woodland and sand forest habitats and the

NGR border consisting of the Usuthu River. Both reserves are fenced off from the surrounding livestock areas, where communal subsistence farming is practised. With the exception of the eastern border of NGR, the fences are well maintained and effective at preventing wildlife-livestock contact; with the TEP fence electrified to contain the elephants.

Sampling and laboratory testing

Serum samples were collected opportunistically during routine culling of nyala and impala antelope for population control purposes in both reserves between June 2016 and May 2018. Animals were harvested by park management at night using a spotlight and rifle and blood was collected into plain Vacutainer® tubes during exsanguination. The geographic co-ordinates of the collection site and the species, sex and age category of the animal were recorded; age was classified by harvesters, based on size, coat colour, horn development and dentition, as juvenile (12 to a maximum of 24 months), sub-adult (up to 3-4 years) and adult (>4 years). Serum was separated by centrifugation in a field laboratory at TEP and stored at -20°C before being transported in a portable freezer at -20°C to Pretoria under a KZNDARD permit. On arrival, sera were inactivated at 56°C for 1 hour in a water bath to minimize the risk of foot-and-mouth disease virus contamination and stored at -20°C until used. The serum neutralization test was used and is a gold standard for RVFV serodiagnosis (OIE, 2012). The serum neutralization test was performed in 96-well plate (AEC-Amersham) format according to the standard protocol of the Virology Section, Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria which follows the method prescribed by the World Organization for Animal Health (OIE, 2012). Briefly: Sera were diluted 1:5 in PBS+ (phosphate buffered saline with added MgCl and CaCl) and two-fold dilutions of the serum were made. The $TCID_{50}$ was determined using the Karber method (Karber, 1931). A volume of 100 $TCID_{50}$ virus (Smithburn vaccine strain) was added to each dilution and incubated at 37°C for 60 min. A total of 80 µl of African green monkey kidney cells (Vero)

(480,000 cells/ml) in MEM containing 5% foetal calf serum (Biowest, Celtic) was added to each well. The microplates were incubated at 37°C in an atmosphere containing 5% CO_2 and observed daily for cytopathic effect. The titre was calculated as the dilution at which 50% of the cells were affected. Results were only accepted if all controls gave the expected results (virus control, positive serum and negative serum). A serum dilution of ≥1:10 was used to define seropositivity.

Statistical analysis

The period prevalence of seropositivity to RVFV in antelope sera was calculated, overall and by sampling site, species, sex, age group (juvenile, sub-adult, adult), collection year (June 2016 to May 2017 vs. June 2017 to June 2018) and proximity to a floodplain or swamp (≤2 km vs. >2 km). Locations of sample collection sites, water sources (NFEPA, 2016) and reserve boundaries (EKZN Wildlife, 2015) were plotted in ArcGIS 10.2 (Esri Corporation, Redlands, CA, U.S.A.) and distance between sampling points and floodplains or swamps was calculated in ArcGIS based on the water source shapefile (NFEPA, 2016). Univariable associations were assessed using Fisher's exact test and predictor variables associated with RVFV seropositivity ($P < 0.2$) were considered for multivariable analysis after checking for collinearity using Pearson's *r*. A multivariable logistic regression model was used to estimate the association between seropositivity to RVFV and site, species, sex, age, year and proximity to a floodplain or swamp, while controlling for confounding. All two-way interactions were also tested for significance. The fit of the model was assessed using a Hosmer-Lemeshow goodness-of-fit test. Statistical analyses were done using Stata 15 (StataCorp, College Station, TX, U.S.A.) and significance was assessed at $P < 0.05$.

Results

A total of 326 serum samples were obtained at irregular intervals from June 2016 to June 2018; most were from TEP, with only 20 from NGR, all during August-December 2016 (Figure 1). The seroprevalence over the collection period was 35.0% (114/326; 95% CI: 29.8-40.4%) (Table 1) and, although somewhat higher for NGR, did not differ significantly between the reserves ($P = 0.087$). There was also no significant difference in seroprevalence between nyala (97/289; 33.5%; 95% CI: 21.0-33.0%) and impala (17/37; 45.9%; 95% CI: 28.1-39.3%) ($P = 0.146$). Amongst the youngest animals (juveniles <2 years old), seroprevalence was 28.6% (95% CI: 11.3-52.2%), including 4 seropositive animals harvested during October-December 2017 and one in May 2018.

After adjustment for confounding, the multivariable model (Table 2) showed that animals that were sampled within 2 km of a floodplain or swamp were more likely to be seropositive (OR = 3.30; 95% CI: 1.70-6.38; $P < 0.001$). This pattern is visible in the distribution of positive and negative samples in TEP but is not clear for NGR (Fig. 1). Odds of seropositivity was the lowest in sub-adults and was significantly higher both in juveniles (OR = 4.73; 95% CI: 1.30-17.3; $P = 0.019$) and in adults (OR = 3.98; 95% CI: 1.83-8.67; $P = 0.001$). Males were more likely than females to be seropositive (OR = 2.66; 95%CI: 1.51-4.68; $P = 0.001$). No two-way interactions between predictors were significant and were therefore not included in the model. The Hosmer-Lemeshow goodness-of-fit statistic indicated adequate model fit ($P = 0.528$). Restricting the analysis to samples from TEP only, resulted in no material change in the effects of the other predictors.

Discussion

This study reports the seroprevalence of antibodies to RVFV in wild antelope species in NGR and TEP, two reserves adjacent to livestock farming areas in which a high rate of RVFV

circulation has recently been reported (Van den Bergh et al., 2019). The overall seroprevalence in nyala and impala was fairly similar to that in cattle and goats. This pattern was also evident in Kenya, where the seroprevalence of RVFV in wildlife increased in parallel with domestic animals during a major outbreak of disease in livestock, and similarly declined afterwards (Britch et al., 2013). Livestock in our study area may graze along the fences of the reserves but, with the exception of a portion of the eastern fence of NGR, they are not able to enter the reserves. Direct transmission from animal to animal is only possible when animals can lick each other or aborted foetuses (Pepin, Bouloy, Bird, Kemp, & Paweska, 2010). The most likely explanation for the apparently similar seroprevalence in both domestic and wild ruminant populations is that they are all part of the same vector-host maintenance system, including one or more mosquito species without strict host preferences. The definition of a reservoir depends on specifying the target population (Haydon et al., 2002); therefore, it is possible that the wildlife-vector system may act as a reservoir for livestock, or the livestock-vector system may act as a reservoir for wild ruminants. In terms of the potential health risk to humans, all three components (livestock, wildlife and vector) may constitute the reservoir, with livestock likely being the most important source population for human infection; although in our study area, where clinical cases are not reported, this remains to be determined.

Antibodies to RVFV have been detected in multiple wildlife species in Africa and the Middle East. In South Africa low level circulation among buffaloes has been recorded during inter-epidemic periods (LaBeaud et al., 2011; Beechler et al., 2015) and it has been suggested that wildlife may play an important role in the survival cycle of the virus during these periods (Olive et al., 2012). A number of ungulates have been recorded as susceptible to clinical disease due to RVFV (Evans et al., 2008; DAFF, 2011; Olive et al., 2012; Pienaar & Thompson, 2013), but no further investigation has been done on the transmission efficiency

of ungulates or other wildlife. It is also unknown whether RVF causes any disease or has any detrimental effect on wildlife in the area; the only previously reported occurrence of RVF in nyala was death of a single animal during the outbreak in 2010 on a farm in the Northern Cape, well outside the species' natural range (DAFF, 2011). However, any sporadic clinical cases, particularly abortions, that may occur in wildlife in the study area would likely remain undiagnosed or undetected because of the environment.

Silent circulation of the virus in livestock has been described in adjacent Mozambique, where only a few outbreaks have been reported despite widespread serological evidence of exposure to RVFV in livestock and African buffalo (Fafetine et al., 2013; Moiane et al., 2017). With recent evidence of active RVFV circulation in livestock (Van den Bergh et al., 2019) and wild antelope species (this study) in northern KZN, it is evident that the virus may circulate for long periods on the tropical coastal plain of south-eastern Africa with few or no reported outbreaks or diagnosis of clinical cases in humans or animals. In contrast to the KNP, where a lower seroprevalence (generally <10%, based on the serum neutralization test) and low seroconversion rate was found in African buffaloes (Beechler et al., 2015) and it was concluded that a combination of horizontal and vertical (transovarial) transmission by mosquitoes was necessary for RVFV maintenance (Manore & Beechler, 2015), a high seroprevalence was found in this study. Therefore, the role of, and necessity for, transovarial transmission by mosquitoes in the maintenance of RVFV circulation in such tropical lowland areas requires further investigation.

In this study the seroprevalence between the two years was different, with the first year being higher than the second. Seroprevalence may be expected to change over time if the rate of seroconversion changes, which was shown to be the case in the livestock in the adjacent farming areas; the rate of change of seroprevalence depends also on the rate of sero-reversion (Muench, 1959) which is unknown. However, it is difficult to know whether the apparent

difference between years reflected a real change in seroprevalence, since animals were sampled by convenience and by different people over time, likely using different criteria for selection. The apparent difference in seroprevalence between the two reserves was not significant in the multivariable model and was likely an artefact due to the small sample size from NGR and due to confounding, since all the samples from NGR were collected during the first year.

There was a clear and significant positive association between RVFV seroprevalence and proximity of sampling site to surface water, namely the Muzi swamp in TEP. A cut-off of 2 km was selected, using the approximate average maximum distance (2214 m) that *Culex* (*Cux.*) *tritaeniorhynchus* would fly in order to find a blood meal (Verdonschot & Besse-Lototskaya, 2014). This was found to be the most abundant mosquito species caught during an entomological study in the same area (unpublished data) and has been shown to be a competent RVFV vector elsewhere (Jupp et al., 2002). Similar observations were made in Mayotte where people and animals were more likely to have antibodies to RVFV when they were located near a water source (Lernout et al., 2013), likely due to more frequent exposure to vectors.

It is noteworthy that males of both species were more likely to be seropositive than females. Impala males tend to have a larger home range than females (Vincent, 1979); however, this is not the case for nyala. Males of both species, but particularly nyala, are also much larger, and seroprevalence is reported to increase with size in cattle (Jeanmaire et al., 2011). It has also been observed in primates that larger animals are more likely to be infected with malaria, presumably due to their higher production rate of chemical attractants (Davies, Ayres, Dye, & Deane, 1991). Apart from carbon dioxide, these include kairomones and volatile organic compounds such as ketones and acetones that accumulate in blood of ruminants after feeding, and that are used by haemotophagous Diptera to detect the target when searching for a blood

meal (Clements, 1999). The much larger volume of air exhaled by male antelope such as nyala may therefore significantly increase their attractiveness to mosquitoes. Other factors may be increased skin surface area and thermal radiation, and differences in visual stimulation (Clements, 1999) due to the difference in size and colour between male and female nyala; however, further research would be required to confirm this for nyala.

It is unclear why seroprevalence was higher in juveniles than in sub-adults, although the low number of juveniles sampled, and their non-random selection may have reduced the accuracy of the estimate. For most infectious diseases, with a constant rate of exposure, it is expected that there will be a gradual increase in seroprevalence with increasing age, whereas variations in rate of exposure, which is expected with RVFV, will distort this relationship. Another factor that could have influenced this result is possible incorrect age classification during sample collection. Nevertheless, considering the presence of antibodies in juveniles harvested in 2017 and 2018, which were ≤24 months old at the time of collection, it is evident that exposure to RVFV occurred at least until 2016, well after the last major outbreaks in South Africa in 2008-2011. Combining juveniles and sub-adults into a single category resulted in a clear difference in seroprevalence between young animals (22%) and adults (40%) which was significant in a multivariable model (OR = 2.65; P = 0.003). This increase in seroprevalence with age, along with the fact that the seroprevalence was high in all age groups, indicates that RVFV is endemic at high levels in the wildlife population and that wild antelope may be an important component of the RVFV maintenance system in the study area.

Conclusion

This study has demonstrated that recent circulation of RVFV has occurred in antelope in the absence of apparent clinical disease in northern KwaZulu-Natal, South Africa, with

seroprevalence in antelope similar to that reported in domestic ruminants in adjacent areas. It appears that, under similar ecological conditions, domestic and wild ruminants may play a similar role in maintenance of virus circulation, and either or both may serve as the mammalian host in a vector-host maintenance system. However, very little is known about transmission efficiency and susceptibility of wildlife hosts, or the role of transovarial transmission by mosquitoes. The identity and population dynamics of the important vectors and the impact of the presence of the virus on animal and human health should also be further investigated.

Acknowledgements

Conflict of interest statement

No conflicts of interest declared.

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Tables

Table 1: Seroprevalence of Rift Valley fever virus in wild antelope in far northern KwaZulu-Natal: descriptive statistics and univariate associations.

Variable	N	Seroprevalence (%)	95% CI (%)	*P*-value
Sampling site				0.087
NGR	20	55.0	31.5 - 76.9	
TEP	306	33.6	28.4 - 39.3	
Sampling year				<0.001
Year 1 (2016 - 2017)	105	52.4	42.4 - 62.2	
Year 2 (2017 - 2018)	221	26.7	21.0 - 33.0	
Species				0.146
Nyala	289	33.6	28.1 - 39.3	
Impala	37	45.9	29.5 - 63.1	
Sex				0.003
Female	172	27.3	20.8 - 34.6	
Male	154	43.5	35.5 - 51.7	
Age				0.009
Juvenile	21	28.6	11.3 - 52.2	
Sub-adult	65	20.0	11.1 - 31.8	
Adult	240	39.6	33.4 - 46.1	
Proximity to floodplain				<0.001
>2km	217	28.6	22.7 - 35.1	
≤ 2km	77	55.8	44.1 - 67.2	
Total	326	35.0	29.8 - 40.4	

Table 2: Factors associated with seropositivity to Rift Valley fever virus in wild antelope in far northern KwaZulu-Natal: multivariable logistic regression model.

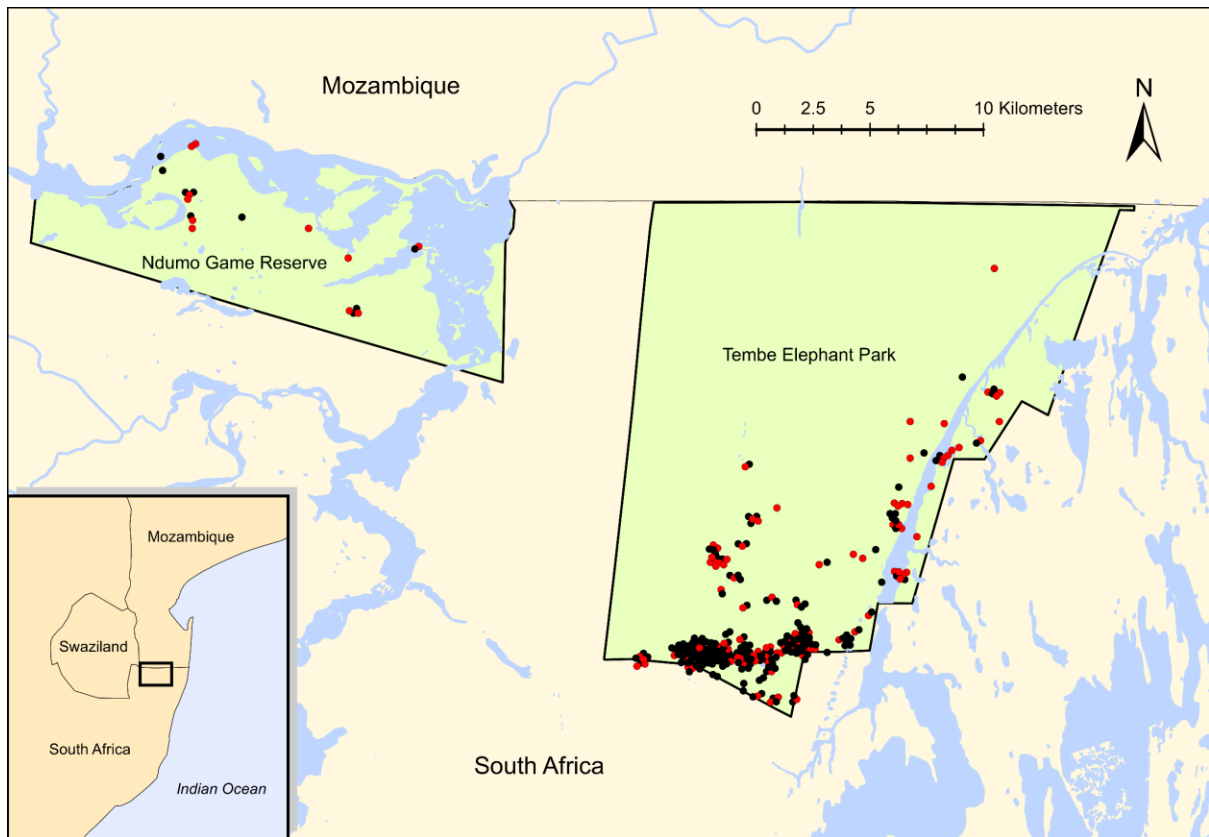| Variable and level	Odds ratio	95% CI	*P*-value
Sampling site			
Ndumo	1*	–	–
Tembe	1.65	0.52 - 5.22	0.396
Sampling year			
Year 1 (2016 - 2017)	1*	–	–
Year 2 (2017 - 2018)	0.40	0.22 - 0.72	0.002
Species			
Nyala	1*	–	–
Impala	1.08	0.48 - 2.40	0.859
Sex			
Female	1	–	–
Male	2.66	1.51 - 4.68	0.001
Age			
Juvenile	4.73	1.30 - 17.3	0.019
Sub-adult	1*	–	–
Adult	3.98	1.83 - 8.67	0.001
Proximity to floodplain			
>2 km	1*	–	–
≤2 km	3.30	1.70 - 6.38	<0.001

* Reference level

Figure 1: A map of Ndumo Game Reserve and Tembe Elephant Park (EKZN Wildlife, 2015), showing rivers, the maximum extent of floodplains and swamps (NFEPA, 2016), and the locations where animals were sampled. Red dots represent RVFV antibody-positive animals and black dots represent seronegative animals; coincident points are slightly dispersed to reduce overlap.