

# SERO-EPIDEMIOLOGICAL SURVEY OF RIFT VALLEY FEVER VIRUS IN RUMINANTS IN NYANDARUA COUNTY, KENYA

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## ABSTRACT

Rift Valley fever (RVF) is a mosquito-borne, viral zoonosis that causes significant economic and public health impacts in ruminant animals and humans. It is primarily transmitted to animals through bites from infected *Aedes spp.*, and to humans through contact with infected animals. It is among the top five priority zoonotic diseases in Kenya. The current study was aimed at determining Rift Valley fever virus seroprevalence in cattle, sheep, and goats in Nyandarua County. A cross-sectional and purposive sampling techniques was adopted in this study. A total of 301 RVF suspect animals were sampled from 16 villages in Olkalou, Kipipiri, and Wanjohi sub-counties. The RVFV IgG and IgM antibodies were detected using multispecies indirect competitive and capture ELISA respectively. Mixed logistic regression determined the association between RVFV seropositivity, sex, age, species, exposure, and village. A total of 164 cattle, 118 sheep, and 19 goats were sampled, and the overall IgG seroprevalence was 31.23% (95% [CI 26.26 - 36.67]). Cattle, sheep, and goats had seroprevalence of 49.39% (81/164) (95% [CI 41.74 - 57.04]), 9.32% (11/118) (95% [CI 4.08 - 14.57]), and 10.53% (2/19) (95% [CI 0.00 - 24.33]) respectively. The prevalence of IgM on all positive IgG was 3.19% (3/94) (95 [CI 0.00 - 6.74]) in cattle. The overall IgG seroprevalence for all-male species was 6.06% (2/33) (95% [CI 0.00 - 14.20]) and all-females 34.33% (92/268) (95% CI 28.64 - 40.01). High seroprevalence and reported cases of abortions suggest subclinical circulation of RVFV in livestock in Nyandarua. These findings provide evidence of RVF disease status. Prevention and control through animal and human surveillance, timely vaccination, and vector control are required.

**Keywords:** Rift Valley fever (RVF) virus, seroprevalence, ruminants, Nyandarua

## INTRODUCTION

Rift Valley fever (RVF) is a vector-borne, viral zoonosis that causes significant economic and public health impacts in ruminant animals and humans (Nguku *et al.*, 2010). Rift Valley fever (RVFV) is a “negative-sense”, single-strand Ribonucleic Acid (ssRNA) *Phlebovirus* in the family *Phenuiviridae*, and the order *Bunyavirales*. It has three segments; L (large), S (small), and M (medium) comprising different structures of the virion. It measures 80 – 120 nanometers (nm) in diameter and has “a non-structural protein (NSs)”, the virulence element which enables it to cause infections in humans and animals (Kainulainen *et al.*, 2016).

RVF was first reported in 1912 on a farm in Naivasha, Kenya, as hepatic necrosis in sheep and isolated in the laboratory in 1931 (Daubney *et al.*, 1931; McMillen and Hartman, 2018). It occurs in cyclic inter-epidemics of 8-10 years (Breiman, 2010). It usually occurs sporadically after heavy rainfall and flooding experienced during El Niño–Southern Oscillation (ENSO) in endemic areas (Bhardwaj, 2013; Anyamba *et al.*, 2010). The disease is endemic in tropical Africa, including regions in sub-tropical climates such as Madagascar and the Arab Peninsula (Pal *et al.*, 2012). The virus isolation and serological evidence show that the RVF burden in half of the African continent is confined to East and South Africa. The epizootic and epidemics are reported in some countries, including Saudi Arabia and Yemen (Nanyingi *et al.*, 2015).

An increased mosquito population, especially *Aedes*, *Culex*, and *Mansonia* species, after heavy rainfall remains a risk factor for the RVF outbreak (Munyua *et al.*, 2016). The virus is maintained in the dormant eggs of *Aedes* in the soil and hatches 1-2 days after flooding. The emerging

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females *Aedes* are the primary vectors that bite and infect susceptible ruminants. The secondary vectors, *Culex* and *Mansonia* species, become the dominant population 30–40 days later. (Hightower *et al.*, 2012; Linthicum *et al.*, 2016). Exposure to sick animals' materials and secretions results in human infections though there is no documentation of human-to-human transmission ( Daubney *et al.*, 1931; Anyamba *et al.*, 2010).

Most RVF serological surveillance in Kenya has focused on areas considered high risk and likely to experience epidemics ( Munyua *et al.*, 2016; Hassan *et al.*, 2020). There is limited research to determine RVFV transmission in low-endemic areas like Nyandarua (Gray *et al.*, 2015). The study's objective was to determine the extent of RVFV exposure in cattle, sheep, and goats in Nyandarua County, Kenya. Targeted sampling was used during the surveillance to detect the emergence of RVF. The method is mainly used to show disease freedom for trade purposes and to detect and monitor endemic diseases ( Willeberg *et al.*, 2012 and Hattendorf *et al.*, 2017).

## MATERIAL AND METHODS

### Study area

The study was conducted in Nyandarua County, which covers an area of 3,285.7 Km<sup>2</sup> and is situated in Central Kenya. To the north, it borders Laikipia County and Nyeri and Murang'a Counties to the North East and the East, respectively. To the south, it borders Kiambu County and Nakuru County to the West. It has a population of 638,289 ( 315,022 males and 323,247 females) living in 179,686 households, and it has an average of 3.5 people per household with a population density of 194 people per Km<sup>2</sup> (Population and Census, 2019). The average temperatures range from 2 °C to 25 °C with rainfall between 700 -1,500 mm annually. Nyandarua County has Lake Ol Bolosat providing a conducive environment for potential RVF virus vectors' emergence.

### Study design and sample size determination

A cross-sectional study design was used in Nyandarua County, where the sample size was determined based on the risk-based targeted sampling of the suspect and contact herd livestock. The suspect livestock included all animals with recently reported cases of abortion, and livestock contacts in the same herd. This sampling method utilizes risk-based veterinary surveillance as the primary objective

and safeguards the health of the animals with minimal available resources. There was a higher probability of disease detection in a definite section of the population of interest which ensured public health and an impact on trade (Stärk *et al.*, 2006). Based on the risk-based targeted purposive sampling, all RVF clinical suspects' livestock and contact herds were included during the passive surveillance between July 2020 and January 2021 and screened for RVF virus for IgG and IgM antibodies (Doherr *et al.*, 2001). The number of villages and serum collected was not based on statistical consideration but on the reported abortion cases (Sindato *et al.*, 2015). A total of 301 blood sera from livestock that had recently experienced abortion and animals in the same herd were collected for laboratory analysis.

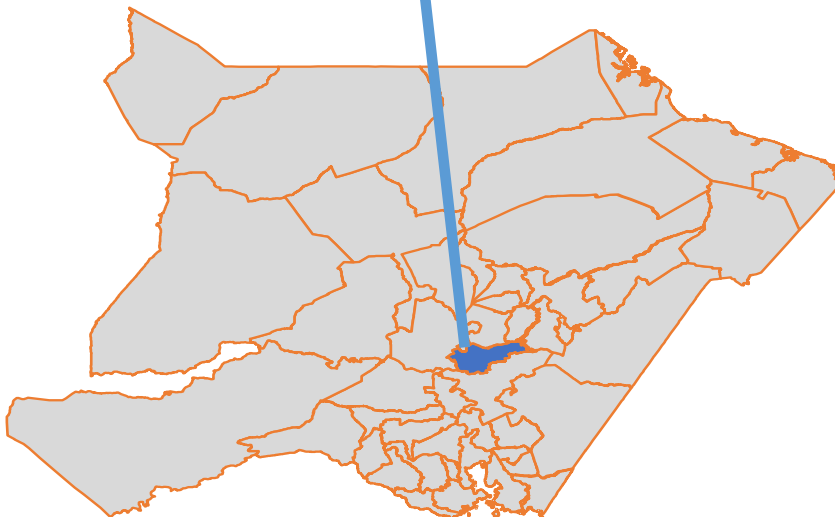
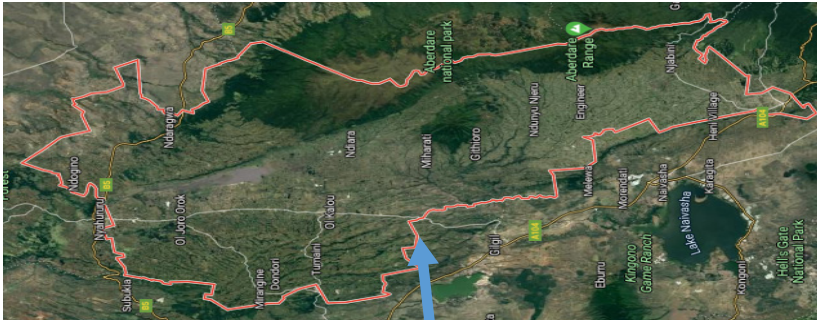
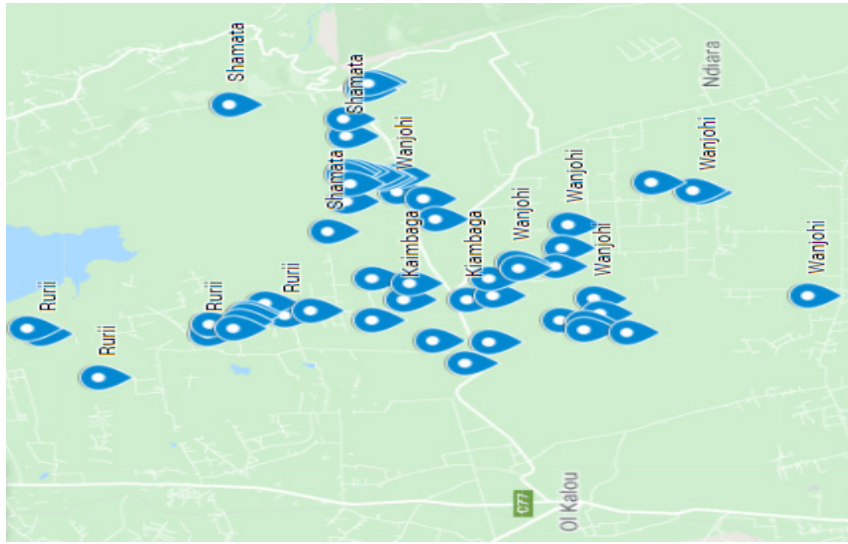
### Selection of the sites and sampling of animals

In July 2020 and January 2021, samples were collected in three sub-counties of Olkalou, Kipipiri, and Ndaragwa in Nyandarua County. Samples included blood in plain tubes with a clot activator for reported cases of abortion in goats, sheep, and cattle during the study period. Sampling was conducted in four wards in Rurii and Kaimbaga, located in Olkalou Subcounty, Wanjohi, in Kipipiri Subcounty, and Shamata in Ndaragwa Subcounty. These wards border each other, and zero-grazing is the predominant livestock production system characterized by confining the animals within the compound.

Sixteen villages were sampled and comprised 57 livestock farmers (Table I). Targeted sampling was done from suspect and contact herd livestock and it determined the final sample size. Three hundred and one (301) livestock blood sera were collected from 57 herds that had not been vaccinated against RVF to avoid detection of false anti-RVFV positive antibodies. County veterinarian and animal health technician assisted in identifying animals that had a recent abortion for inclusion in targeted sampling during the study.

### Blood sample collection

Five millilitres (5mls) of whole blood were collected from the animals by jugular venipuncture into plain vacutainer tubes for serological analysis. The samples were linked to the animal per respective homestead. . The blood was collected from animals with a recent history of abortion, contact herd, and sick animals within the homestead.



**Figure 1.** Animal Sampling Sites in Nyandarua County

Serum was then extracted through centrifugation at a makeshift laboratory in the field. After serum separation, samples were transferred into 2mls cryovials tubes and transported in cooler boxes with ice cubes to Central Veterinary Laboratory in Kabete, where they were stored at -80 °C until testing. The study location geocodes were collected electronically using the Epicollect5 mobile

application (Aanensen, *et al.*, 2009). The standardized and pre-tested questionnaires were accessed through smartphones and administered to key informants, primarily livestock owners, to collect data on herd size, recently reported cases of animal abortion, contact herd, history of livestock vaccination, animal age and sex, and presence of mosquito vector.

TABLE I - DISTRIBUTION OF ANIMALS SAMPLED BY VILLAGE, SPECIES, CLINICAL HISTORY, AND AGE IN NYANDARUA COUNTY

Study village	Species	Number sampled		Aborted	Clinical history Contact herd	Age (Year)		Total
		Male	Female			≤ 1	> 1	
Gichungo	Cattle	0	23	10	13	0	23	23
	Sheep	2	50	1	51	10	42	52
	Goats	0	0	0	0	0	0	0
Mugathika	Cattle	0	13	3	10	0	13	13
	Sheep	0	2	2	0	0	2	2
	Goats	0	0	0	0	0	0	0
Mukindu	Cattle	2	6	5	3	0	8	8
	Sheep	0	3	0	3	0	3	3
	Goats	0	0	0	0	0	0	0
Kiaduba	Cattle	2	7	3	6	3	6	9
	Sheep	0	0	0	0	0	0	0
	Goats	0	0	0	0	0	0	0
Kanjogu	Cattle	6	30	5	31	21	15	36
	Sheep	0	5	0	5	2	3	5
	Goats	2	17	1	18	1	18	19
Kahindu	Cattle	0	5	3	2	0	5	5
	Sheep	0	0	0	0	0	0	0
	Goats	0	0	0	0	0	0	0
Michore	Cattle	6	23	7	22	2	27	29
	Sheep	0	9	5	4	0	9	9
	Goats	0	0	0	0	0	0	0
Kirima	Cattle	0	1	1	0	0	1	1
	Sheep	2	6	2	6	2	6	8
	Goats	0	0	0	0	0	0	0
Huherio	Cattle	1	16	5	12	1	16	17
	Sheep	0	0	0	0	0	0	0
	Goats	0	0	0	0	0	0	0
Malewa	Cattle	0	4	4	0	0	4	4
	Sheep	0	0	0	0	0	0	0
	Goats	0	0	0	0	0	0	0
Magomano	Cattle	1	4	2	3	0	5	5
	Sheep	2	15	2	15	3	14	17
	Goats	0	0	0	0	0	0	0
Gichigirira	Cattle	1	8	3	6	1	8	9
	Sheep	5	13	0	18	11	7	18
	Goats	0	0	0	0	0	0	0
Miti tano	Cattle	0	2	1	1	0	2	2
	Sheep	1	3	0	4	0	4	4
	Goats	0	0	0	0	0	0	0
Kiburuti	Cattle	0	1	1	0	0	1	1
	Sheep	0	0	0	0	0	0	0
	Goats	0	0	0	0	0	0	0

Mubao	Cattle	0	1	1	0	0	1	1
	Sheep	0	0	0	0	0	0	0
	Goats	0	0	0	0	0	0	0
Ndemi	Cattle	0	1	1	0	0	1	1
	Sheep	0	0	0	0	0	0	0
	Goats	0	0	0	0	0	0	0
<b>Total</b>		<b>33</b>	<b>268</b>	<b>68</b>	<b>233</b>	<b>57</b>	<b>244</b>	<b>301</b>

## Serological testing

### IgG analysis

A commercial indirect competitive enzyme-linked immunosorbent assay (Rift Valley Fever Competition Multi-species, cELISA, ID Screen; IDVet Innovative Diagnostics, Grabels, France) was used for testing according to the manufacturer's instructions. Briefly, 50  $\mu$ L of prediluted serum samples and both controls (Positive and Negative) were added to the coated wells of the plate, then 50  $\mu$ L of RVF sample dilution buffer was added to each well. The plate was incubated at 37 °C for 1 hour after covering it with an adhesive cover. The plate was washed three times with 300  $\mu$ L of wash solution, and 100  $\mu$ L of the conjugate was added and incubated at 21 °C for 30 minutes. The wash step was repeated three times and 100  $\mu$ L of substrate solution was added to each well, followed by incubation at 21 °C for 15 minutes in the dark. Finally, add 100  $\mu$ L stop solution and read the results at 450 nm using a microplate reader (HALO LED 96 Microplate Reader).

Validation of the test was done when the mean value of the Negative Control Optical Density was greater than 7 ( $OD_{NC} > 0.7$ ) and that of the Positive Control ( $OD_{PC}$ ) was < 30% of the  $OD_{NC}$  ( $OD_{PC}/OD_{NC} < 0.3$ ). Interpretation of the test for each sample was calculated as the competition percentage (S/N %);  $S/N\% = OD_{Sample} / OD_{NC} \times 100$ . The values of less than or equal to ( $\leq$ ) 40% were considered positive, greater than (<) 40%, and less than or equal to ( $\leq$ ) 50% were indeterminate, and greater than (>)50% were considered negative (Ellis *et al.*, 2014).

### IgM analysis

The positive serum samples for RVFV with IgG antibodies were then tested for IgM antibodies using the IgM capture

enzyme-linked immunosorbent assay (Rift Valley Fever IgM Capture, ID Screen; IDVet Innovative Diagnostics, Grabels, France), and 10  $\mu$ L of prediluted serum samples and both controls (Positive and Negative) were added to the coated wells of the plate, then 40  $\mu$ L dilution buffer added to each well. The plate was incubated at 37 °C for 1 hour after covering it with an adhesive cover. The plate was washed three times with 300  $\mu$ L of wash solution, 50  $\mu$ L of the RVFV nucleoprotein added to the even-numbered columns only, and 50  $\mu$ L of dilution buffer to odd-numbered columns. The 50  $\mu$ L of the conjugate was added and incubated at 37°C for 1 hour. The wash step was repeated three times, and 100  $\mu$ L of substrate solution was added to each well, followed by incubation at 21 °C for 15 minutes in the dark. Finally, add 100  $\mu$ L stop solution and read the results at 450 nm using a microplate reader (HALO LED 96 Microplate Reader).

The Optical density was read and interpreted differently from that of IgG analysis. The test validation was done by calculating the net Optical Density (OD) results (net  $OD = OD_{even\ well} - OD_{odd\ well}$ ). The test was validated if; the mean value of the net Positive Control OD ( $OD_{PC}$ ) was greater than 0.350, the ratio of the mean values of the net Positive and Negative Control ODs (net  $OD_{PC}$  and net  $OD_{NC}$ ) is greater than 3 (absolute value of  $OD_{NC}$ ); net  $OD_{PC}/net\ OD_{NC} > 3$ . Interpretation of the test for each sample was calculated as the S/P percentage (S/P%);  $S/P\% = net\ OD_{Sample} / net\ OD_{CP} \times 100$ . The samples presenting S/P% values of less than or equal to ( $\leq$ ) 40% were considered negative, between 40% and 50% were considered doubtful, and greater than or equal to ( $\geq$ )50% were considered positive.

### Statistical analysis

The univariable logistic regression analysis was done to determine the link between the putative risk factors and IgG serostatus for each livestock. The risk factors analysed

were exposure, species, age, and sex. The effects of the exposure variables on the distribution of the outcome variable while controlling for other variables (covariates), were estimated. Variables for which an association ( $p \leq 0.02$ ) was detected were included as predictors in a generalized mixed effect multivariable logistic regression model.

The factors meeting this measure were exposure (abortion/contact herd), sex, and species. Using a backward stepwise elimination process, the factors and their interactions were assessed and retained them in the model only when  $p \leq 0.05$ . Finally, the fitted individual's models were evaluated by including the study village as a random adjust clustering effects of RVF IgG seropositivity to check the likelihood ratio among seropositive livestock in each village was estimated. The adjusted odds ratios (ORs) for IgG seropositivity were estimated using logistic regression using epiInfo7. All analyses were performed using EpiInfo7 software (Version 7.2.2.6) and MS Excel (2013).

### Ethical approval

This study's ethical approval was granted by KNH-UON ERC (Ref: KNH-ERC/A/373), and field approval for the study was given by both the Directorate of Veterinary Services in Kenya and Nyandarua county Director of Veterinary Services. Individual informed consent was required for this investigation, and all farmers' data were assigned unique identification and anonymised for confidentiality.

## RESULTS

### RVFV IgG seroprevalence

A total of 301 livestock were sampled and analysed. The overall RVFV antibody seroprevalence was 31.23% (95% [CI 26.26 - 36.67]) for IgG. The seropositivity for cattle was 49.39% (81/164) (95% [CI 41.74 - 57.04]), sheep 9.32% (11/118) (95% [CI 4.08 - 14.57]), and goats 10.53% (2/19) (95% [CI 0.00 - 24.33]) (Table II).

TABLE II - RIFT VALLEY FEVER IGG SEROPREVALENCE BY STUDY VILLAGE AND LIVESTOCK SPECIES IN NYANDARUA COUNTY

Study village	Cattle			Sheep			Goat		
	Pos	SP (%)	95% (CI)	Pos	SP (%)	95% (CI)	Pos	SP (%)	95% (CI)
Gichungo	13	7.93	3.79-12.06	4	3.39	0.12- 6.66	0	0	-
Mugathika	7	4.27	1.17 - 7.36	0	0	-	0	0	-
Mukindu	5	3.05	0.42 - 5.68	0	0	-	0	0	-
Kiaduba	5	3.05	0.42 - 5.68	0	0	-	0	0	-
Kanjogu	10	6.10	2.44 - 9.76	0	0	-	2	10.53	0.00-24.33
Kahindu	2	1.22	0.00 - 2.90	0	0	-	0	0	-
Michore	11	6.71	2.88- 10.54	0	0	-	0	0	-
Kirima	1	0.61	0.00 - 1.80	2	1.69	0.00- 4.02	0	0	-
Huherio	12	7.32	3.33 - 11.30	0	0	-	0	0	-
Malewa	4	2.44	0.08 - 4.80	0	0	-	0	0	-
Magomano	2	1.22	0.00 - 2.90	4	3.39	0.12- 6.66	0	0	-
Gichigirira	5	3.05	0.42 - 5.68	0	0	-	0	0	-
Miti tano	1	0.61	0.00 - 1.80	1	0.85	0.00- 2.50	0	0	-
Kiburuti	1	0.61	0.00 - 1.80	0	0	-	0	0	-
Mubao	1	0.61	0.00 - 1.80	0	0	-	0	0	-
Ndemi	1	0.61	0.00 - 1.80	0	0	-	0	0	-
<b>Total</b>	<b>81</b>	<b>49.39</b>	<b>41.74-57.04</b>	<b>11</b>	<b>9.32</b>	<b>4.08-14.57</b>	<b>2</b>	<b>10.53</b>	<b>0.00-24.33</b>



The highest IgG seroprevalence in cattle per village was in Gichungo at 7.93% (13/164), followed by Huherio at 7.32% (12/164). In sheep, it was 3.39% (4/118) observed in two villages of Gichungo and Magomano (Table II). The overall IgG seroprevalence for all-male species was 6.06% (2/33) (95% [CI 0.00 – 14.20]) and for all females was 34.33% (92/268) (95% [CI 28.64 - 40.01]). The female cattle had an IgG seroprevalence of 54.48% (95% [CI 46.01 - 62.77]) whereas male cattle had 10.53% (95% [CI 1.30-33.14]). Female sheep had an IgG seroprevalence of 10.38 % (95% CI 5.30-17.81), while female goats had an IgG seroprevalence of 11.76 % (95% CI 1.46-36.44). Cattle that had recent cases of abortion had IgG seroprevalence of 80.70% (95% CI 68.09-89.95) compared to the contacts herd with 32.71% (95% CI 23.95-42.45). The IgG seroprevalence of aborted sheep was 41.67% (95% CI 15.17-72.33), and the contact herd sheep was 5.66% (95% CI 2.11-11.91). Only one goat with a recently reported case of abortion tested negative for IgG, and the contacts herd seroprevalence was 11.11% (95% CI 1.38-34.71). The cattle > 1 year old had IgG seroprevalence of 48.53% (95% [CI 39.88-57.25]) whereas sheep and goats > 1 year old had an IgG seroprevalence of 2.22% (95% [CI 0.27-7.80]) and 11.11% (95% [CI 1.32-34.71]) respectively. Cattle, sheep, and goats ≤ 1-year-old had IgG seroprevalence of 53.57% (95% [CI 33.87-72.49]), 32.14% (95% [CI 15.88-52.35]), and 0% respectively as indicated in Table III.

TABLE III - RIFT VALLEY FEVER SEROPREVALENCE IN CATTLE, SHEEP, AND GOATS BY SEX, EXPOSURE, AND AGE

Animal variables	Animal Sampled	IgG Positive	IgG SP (CI)	IgM Positive	IgM SP (CI)
<b>Cattle</b>					
<i>Sex</i>					
Female	145	79	54.48 (46.01-62.77)	3	3.19 (0.66 - 9.04)
Male	19	2	10.53 (1.30-33.14)		
<i>Exposure</i>					
Aborted	57	46	80.70 (68.09-89.95)	2	5.26 (0.64 - 17.75)
Contact	107	35	32.71 (23.95-42.45)	1	6.67 (0.17-31.95)
<i>Age (year)</i>					
> 1	136	66	48.53 (39.88-57.25)	2	2.60 (0.32 - 9.07)
≤ 1	28	15	53.57 (33.87-72.49)	1	4.17 (0.11 – 21.12)
<b>Sheep</b>					
<i>Sex</i>					
Female	106	11	10.38 (5.30-17.81)		
Male	12	0	0.00		
<i>Exposure</i>					
Aborted	12	5	41.67 (15.17-72.33)		
Contact	106	6	5.66 (2.11-11.91)		
<i>Age (year)</i>					
> 1	90	2	2.22 (0.27-7.80)		
≤ 1	28	9	32.14 (15.88-52.35)		
<b>Goats</b>					
<i>Sex</i>					
Female	17	2	11.76 (1.46-36.44)		
Male	2	0	0.00		
<i>Exposure</i>					
Aborted	1	0	0.00		
Contact	18	2	11.11 (1.38-34.71)		
<i>Age (year)</i>					
> 1	18	2	11.11 (1.32-34.71)		
≤ 1	1	0	0.00		

**Host aspects influence IgG seropositivity**

A mixed-effect logistic regression was used to investigate RVFV IgG antibody prevalence association with host factors of age, exposure, sex, and species. The exposure (either recent abortion or contact herd) with a  $p = 0.0000$  OR 0.1600 (95% CI 0.0815-0.3142), sex (either male or female) with a  $p = 0.0163$  OR 0.1586 (95% CI 0.0353-0.7129), and species  $p = 0.0000$  OR 0.1726 (95% CI 0.0924-0.3223), and the species (either cattle, sheep or goats) with a  $p = 0.0000$  OR 0.1726 (95% CI 0.0924-0.3223) all were statistically significant and showed association with IgG positive results. Age was not significant ( $p = 0.3112$ ) and no association with IgG positive results with an OR 0.6868 (95% CI 0.3320-1.4210) (Table IV).

The odds of abortion were higher in IgG positive compared to IgG Negative (OR = 11.74; 95% CI 6.30 – 21.87), the odds of female compared to male (OR = 8.01; 95% CI 1.90 – 34.61), while the odds of age > 1 compared to  $\leq 1$  (OR = 0.55; 95% CI 0.31 – 1.00). Seropositivity increased with reported cases of abortion. Animals with a history of recent abortion had an 11-fold likelihood of being seropositive than animals within the same herd without abortion. Meanwhile, females had an 8-fold likelihood compared to males.

TABLE IV- EFFECT OF AGE, EXPOSURE, SEX, AND SPECIES ON RIFT VALLEY FEVER VIRUS SEROPOSITIVITY IN NYANDARUA

Variable	Odds Ratio	RVF Seroprevalence	
		95% CI	p-Value
Age (> / $\leq$ )	0.6868	0.3320 - 1.4210	0.3112
Exposure (contact/abortion)	0.1600	0.0815 - 0.3142	0.0000 <sup>a</sup>
Sex (male/female)	0.1586	0.0353 - 0.7129	0.0163 <sup>a</sup>
Species	0.1726	0.0924 - 0.3223	0.0000 <sup>a</sup>
Age > 1	0.5532	0.3053 - 1.0024	-
Abortion	11.7356	6.2984 - 21.8665	-
Female	8.1023	1.8968 - 34.6101	-

<sup>a</sup> Significance level : CI; Confidence Interval, RVF; Rift Valley Fever

**RVFV IgM seroprevalence**

Ninety-four (94) IgG-positive sera samples were screened for IgM, and three female cattle were found seropositive for the virus. The animals with anti-RVFV IgM antibodies, an indicator for recent infection, had a seroprevalence of 3.19% (95 CI 0.66 - 9.04) of all IgG-positive sera. Two cattle with ages > 1-year-old represented a seroprevalence of 2.60% (95% CI 0.32 - 9.07), while one cattle  $\leq 1$ -year-old had a seroprevalence of 4.17% (95% CI 0.11 – 21.12). Two of the cattle which turned positive for IgM had

experienced recent abortion (2/3) 5.26% (95% CI 0.64 - 17.75), and one cattle, 6.67% (95% CI 0.17-31.95), was a contact herd (Table III).

**DISCUSSION**

The study provides recent serological evidence of possible RVFV circulation in villages in Nyandarua County, where anti-RVFV antibodies were found in cattle, sheep, and goats populations. This epidemiological shift of RVFV in low-risk areas may be due to Eco climatic changes, vector emergencies, or anthropogenic activities (Munyua *et al.*, 2016; and Rolin *et al.*, 2013). Climatic and environmental drivers may become conducive to the persistence and emergence of potential vectors for the RVFV resulting in outbreaks in the future (Himeidan *et al.*, 2014).

The detection of both IgG and IgM antibodies in the county could be an indicator of recent infection of livestock in the region (Pepin *et al.*, 2010). The replenishment of the livestock herds (Chevalier *et al.*, 2011) may introduce viremic animals in the area ((Rissmann *et al.*, 2020) due to livestock trade (Nicolas *et al.*, 2013) and migration of livestock from the neighbouring areas (Owange *et al.*, 2014). Cattle from Gichungo village had the highest prevalence of 7.93% compared to other villages. In

contrast, sheep from Gichungo and Magamano villages both had the highest prevalence of 3.39%, and only 19 goats were sampled in Kanjogu village and had a prevalence of 10.53%. This may be attributed to farmers' preference in Nyandarua for rearing cattle and sheep for commercial milk and meat production.

Additionally, the disproportionate sampling between the three species may be due to cases of recent abortion reported and less preference for goat rearing. It was the criteria used to collect the targeted sampling for the



study (Gerken *et al.*, 2022). The spatial distribution of seropositivity was spread across all the villages, though farmers practice tethered or zero-grazing within their homestead. This disagrees with findings from earlier studies which have shown that circulation of RVFV is likely to be related to livestock migration (Arum *et al.*, 2015; Lichoti *et al.*, 2014; Nanyingi *et al.*, 2017; Nanyingi *et al.*, 2015; and Owange *et al.*, 2014). There is high RVFV seroprevalence in livestock in other parts of the country, such as Garissa, Marsabit, Wajir, and our findings confirm a high prevalence of RVFV in livestock in Nyandarua, which may serve as an indicator for circulating disease in both livestock and humans (Muiruri *et al.*, 2015; Nanyingi *et al.*, 2015; Nanyingi *et al.*, 2017 and Hassan *et al.*, 2020).

The transmission of RVFV may be spreading to areas that used to be of low endemicity in the past. As per the risk assessment, Nyandarua lies within the proximity of high-risk classified areas of Laikipia and Nakuru (Munyua *et al.*, 2016). The findings compare with another study in the Democratic Republic of Congo (DRC), which showed the presence of RVF in livestock kept by smallholder farmers (Halawi *et al.*, 2019). This study demonstrated that sex, species, and exposure were highly associated with seropositivity. Similar findings have been reported elsewhere (Jeanmaire *et al.*, 2011 and Di Nardo *et al.*, 2014). However, there was no significant influence of the increase in age on seropositivity shown in Nigeria (Alhaji *et al.*, 2020). Animals suspected to have been infected with RVFV and had a history of abortion had and IgG seropositivity. The prevalence in cattle was the highest at 80.70% (46/57) compared to that of sheep at 41.67% (5/12). It may be hypothesized that a relationship between abortions and seropositivity results necessitates further studies. On the other hand, the one goat with a recent history of abortion did not have any anti-RVFV antibodies. The high IgG seroprevalence in  $\leq 1$ -year-old may have resulted from maternal antibodies (Wright *et al.*, 2019).

## CONCLUSION

The high RVFV seroprevalence of IgG and three cases of IgM in susceptible livestock in Nyandarua indicate previous exposure or possible recent infections despite no reported outbreaks during this period. The presence of IgM-positive animals indicates the continuous circulation of the RVFV in unvaccinated livestock in Nyandarua.

Additional surveillance and vaccination programmes are required to mitigate the spread of the RVF virus.

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