Journal of Virological Methods 269 (2019) 70–76

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

A multiplex fluorescence microsphere immunoassay for increased understanding of Rift Valley fever immune responses in ruminants in Kenya

Johanna F. Lindahl[a,b,c,*,1], Izabela K. Ragan[d,e,1], R.R. Rowland[e], Martin Wainaina[a], Deborah Mbotha[a,f], William Wilson[g]

[a] International Livestock Research Institute, Nairobi, Kenya
[b] Zoonosis Science Center, Uppsala University, Uppsala, Sweden
[c] Swedish University of Agricultural Research, Uppsala, Sweden
[d] Department of Biomedical Sciences, Colorado State University, Fort Collins, CO, USA
[e] Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA
[f] Institute for Parasitology and Tropical Veterinary Medicine, Freie Universitaet Berlin, Berlin, Germany
[g] Arthropod-Borne Animal Diseases Research Unit, USDA, ARS, Manhattan, KS, USA

ARTICLE INFO

Keywords:
Serology
Arbovirus
Vector-borne disease
DIVA test
Seroconversion

ABSTRACT

Rift Valley fever virus (RVFV) is an important mosquito-borne pathogen with devastating impacts on agriculture and public health. With outbreaks being reported beyond the continent of Africa to the Middle East, there is great concern that RVFV will continue to spread to non-endemic areas such as the Americas and Europe. There is a need for safe and high throughput serological assays for rapid detection of RVFV during outbreaks and for surveillance. We evaluated a multiplexing fluorescence microsphere immunoassay (FMIA) for the detection of IgG and IgM antibodies in ruminant sera against the RVFV nucleocapsid Np, glycoprotein Gn, and non-structural protein NSs. Sheep and cattle sera from a region in Kenya with previous outbreaks were tested by FMIA and two commercially available competitive ELISAs (BDSL and IDvet). Our results revealed strong detection of RVFV antibodies against the Np, Gn and NSs antigen targets. Additionally, testing of samples with FMIA Np and Gn had 100% agreement with the IDvet ELISA. The targets developed in the FMIA assay provided a basis for a larger ruminant disease panel that can simultaneously screen several abortive and zoonotic pathogens.

1. Introduction

Rift Valley fever virus (RVFV) is a zoonotic, vector-borne pathogen identified in the 1930s in Kenya's Rift Valley region (Daubney et al., 1931; Davies et al., 2003; Findlay, 1932). The virus causes Rift Valley fever (RVF) which is considered an emerging infectious disease that has been reported over most of sub-Saharan Africa, with introduction into the Arabian Peninsula. However, there are competent vectors in other regions, and therefore the concern that the virus has the potential to spread further into Europe and other parts of the world (Chevalier et al., 2010; Moutailler et al., 2008; Turell et al., 2008, 2002).

The virus is known to infect several animal hosts including livestock and wildlife. Animals become primarily infected by mosquito bites, but there is potential for animal-to-animal transmission (Mansfield et al., 2015; Wilson et al., 2018). Infected livestock may lead to abortion storms and high neonatal mortality due to hemorrhages and necrotic hepatitis (Elliott, 1997; Pepin et al., 2010). Humans can get infected by mosquito bites but more commonly by direct contact with infected animal tissues and body fluids (Anyangu et al., 2010). Other less likely transmission routes include vertical transmission (Adam and Karsany, 2008), transmission from mother to child through breastmilk (Nicholas et al., 2014), and aerosols (Hartman et al., 2014). In humans, the disease is usually self-limiting with mild, flu-like symptoms. The disease can progress to hepatic and renal dysfunction as well as disseminated intravascular coagulation (Madani et al., 2003) with as many as 47% of cases becoming fatal (Mohamed et al., 2010).

RVFV can be transmitted by mosquitoes from at least 6 genera (Linthicum et al., 1985; Logan et al., 1991; Pepin et al., 2010; Walter and Barr, 2011). Epidemics have been occurring at 7–10 years intervals in East Africa mostly after extreme weather events, such as excessive rainfall during the El Niño-Southern Oscillation (ENSO) (Métras et al., 2011; Tourre et al., 2009). Heavy rain and flooding results in

* Corresponding author at: ILRI, PO Box 30709, 00100, Nairobi, Kenya.
E-mail address: J.Lindahl@cgiar.org (J.F. Lindahl).
[1] Authors contributed equally.

https://doi.org/10.1016/j.jviromet.2019.04.011
Received 12 February 2019; Received in revised form 7 April 2019; Accepted 7 April 2019
Available online 08 April 2019
0166-0934/

emergence of infected *Aedes* mosquito eggs, which leads to virus transmission to nearby livestock. Additionally, countries such as Kenya are intensifying efforts to utilize irrigation in its expansive arid and semi-arid land (ASAL) regions to mitigate food insecurity and the vulnerability to droughts. Consequently, this brings humans and animals closer to the vectors and increases risk of transmission. As irrigation and agricultural practices continue to expand, the risk of vectors existing even in the dry season also increases. Inter-epidemic seroconversions have recently been observed (Bett et al., 2015, 2017; Mbotha et al., 2017; Sang et al., 2016).

Kenya has experienced multiple outbreaks in the past leading to huge agricultural loss and public health crisis. The heavy rains in 1997 was followed by an outbreak of RVFV with as many as 27,000 humans infected. Farmers reported losing up to 70% of their sheep and goats, and 95% of young lambs (Woods et al., 2002). The next major outbreak was 2006 in Kenya and 2007 in Tanzania (Mohamed et al., 2010). After the 2006–2007 outbreak in Kenya, pastoralists ranked RVFV as the disease with the highest impact on livestock-derived livelihoods (Jost et al., 2010). The losses were estimated to have been more than 30 million USD for the region, but since livestock production is crucial for many individual pastoralists and farmers, the devastating effects for individuals are hard to estimate (Anyamba et al., 2001; Rich and Wanyoike, 2010). Diagnostic assays are valuable tools for surveillance and rapid testing during RVFV outbreaks, but with increasing use of vaccines there are increasing needs to be able to differentiate between the vaccinated and the infected animals. Vaccines like Clone13 are available for use in endemic countries; however, there is no routine vaccination program yet in these countries to use as a control strategy against RVFV.

Diagnostic testing is important for serological screening and molecular detection of RVFV within animal and human populations. Molecular techniques like PCR can offer rapid detection of RVFV during the viremic stages but require specialized equipment and expertise that may not be available in developing countries. Serological assays are of great value to demonstrate freedom of disease and for epidemiological surveys, but there is still need of new tests that are able to differentiate infected from vaccinated animals (DIVA). Two commonly used serological assays for RVFV surveillance and detection during outbreaks are the virus neutralization tests (VNT) and enzyme-linked immunoassays (ELISAs). Virus neutralization tests are highly specific and are the current gold standard for serological testing of RVFV (OIE, 2014). However, the assays must be performed in biocontainment and are time consuming to perform. Enzyme-linked immunoassays offer a rapid and safe method to detect antibodies against RVFV in animals and humans. A major limitation to ELISA use during a RVFV outbreak is the detection of a single analyte at a time, therefore requiring a second test using a different analyte to confirm sample results. Therefore, a high-throughput multiplexing immunoassay that screens and confirms in one test by testing multiple analytes simultaneously is needed for RVFV detection.

Understanding the structural organization of RVFV aids in the development of diagnostic assays that target important viral components and epitopes. The viral genome is tripartite consisting of large (L), small (S) and medium (M) segments. The L segment encodes for the RNA-dependent RNA polymerase. The S segment is an ambi-sense segment that encodes for the nucleocapsid protein (Np) and the nonstructural protein NSs. Np is considered to be highly immunogenic (Fafetine et al., 2007) and has been frequently used in indirect RVFV ELISAs for IgG antibody detection (Jeong et al., 2011; Kim et al., 2012; Paweska et al., 2008, 2007). Therefore, this protein is an ideal target for serological diagnostic development. The NSs protein is a major virulence factor and has been targeted for attenuating the virus (Bird et al., 2008; Nishiyama et al., 2016). An indirect ELISA using recombinant Np and NSs has been developed to be compatible with NSs-deleted vaccine candidates (McElroy et al., 2009). However, there is a variable antibody response to NSs among individuals (Fernandez et al., 2012). Additional evaluation of antibody response to this protein target is warranted in natural infections and from vaccination. Lastly, the M segment encodes for two glycoproteins (Gn, Gc) and two minor proteins, a 78-kDa nonstructural protein and the 14-kDa nonstructural protein (NSm). The glycoproteins elicit the production of neutralizing antibodies and are a correlate of protective immunity (Besselaar and Blackburn, 1991). These proteins have been valuable targets in serological assays to monitor immune protection after vaccination and during natural infections (Faburay et al., 2013; Jäckel et al., 2013). Like Np, NSs and the glycoproteins have been shown to be immunogenetic and offer a means of multiplex detection of RVFV antibodies.

Bead-based multiplexing assays are a versatile, rapid method to simultaneously detect multiple analytes in suspension. The significantly-reduced time and small sample volume needed makes the assay advantageous over traditional serological testing methods during an outbreak (Christopher-Hennings et al., 2013). The most well-known and widely used multiplexing technology, Luminex®, uses dual-colored magnetic polystyrene beads that are covalently coupled to antigen to detect analytes. Because of the multiplexing capability, the bead-based immunoassay has been used to differentiate infected from vaccinated animals for high–consequence viral pathogens such as foot and mouth disease, West Nile virus, and avian influenza (Balasuriya et al., 2006; Clavijo et al., 2006; Watson et al., 2009). A RVFV bead-based suspension assay has been recently validated with experimentally-infected samples for the detection of antibodies and has shown to be DIVA compatible with a candidate RVFV Gn/Gc glycoprotein vaccine (Ragan et al., 2018). This assay included the nucleocapsid Np and the glycoprotein Gn as targets for antibody detection. An evaluation of the fluorescence microsphere immunoassay (FMIA) using field samples is needed to determine the value of the assay in endemic countries.

This study evaluates the FMIA for the detection of RVFV antibodies against Np, Gn, NSm and NSs target antigens with ruminant sera collected in Kenya. The assay was compared to the results from one of two ELISA tests (BDSL and IDvet). The ability of the FMIA to detect IgG and IgM antibodies in sheep and cattle samples is demonstrated. Future applications of the FMIA include developing additional targets to simultaneously detect several ruminant diseases.

2. Methods

2.1. Serum samples

Cattle and sheep serum samples from an area with both previous outbreaks of RVFV as well as documented inter-epidemic transmission (Mbotha et al., 2017) were used for this evaluation (Table 1). These samples were collected under a project where ethical approval was

Table 1
ELISA tested samples included in the FMIA testing.

Study	Species	Negative	Positive	Test	Reference
Cross-sectional	Bovine	183	13	BDSL ELISA	(Bett et al., 2017; Lindahl et al., 2016)
Cross-sectional	Ovine	181	26	BDSL ELISA	(Bett et al., 2017; Lindahl et al., 2016)
Longitudinal	Ovine	19	17*	IDvet ELISA	(Mbotha et al., 2017)

* Including borderline results.

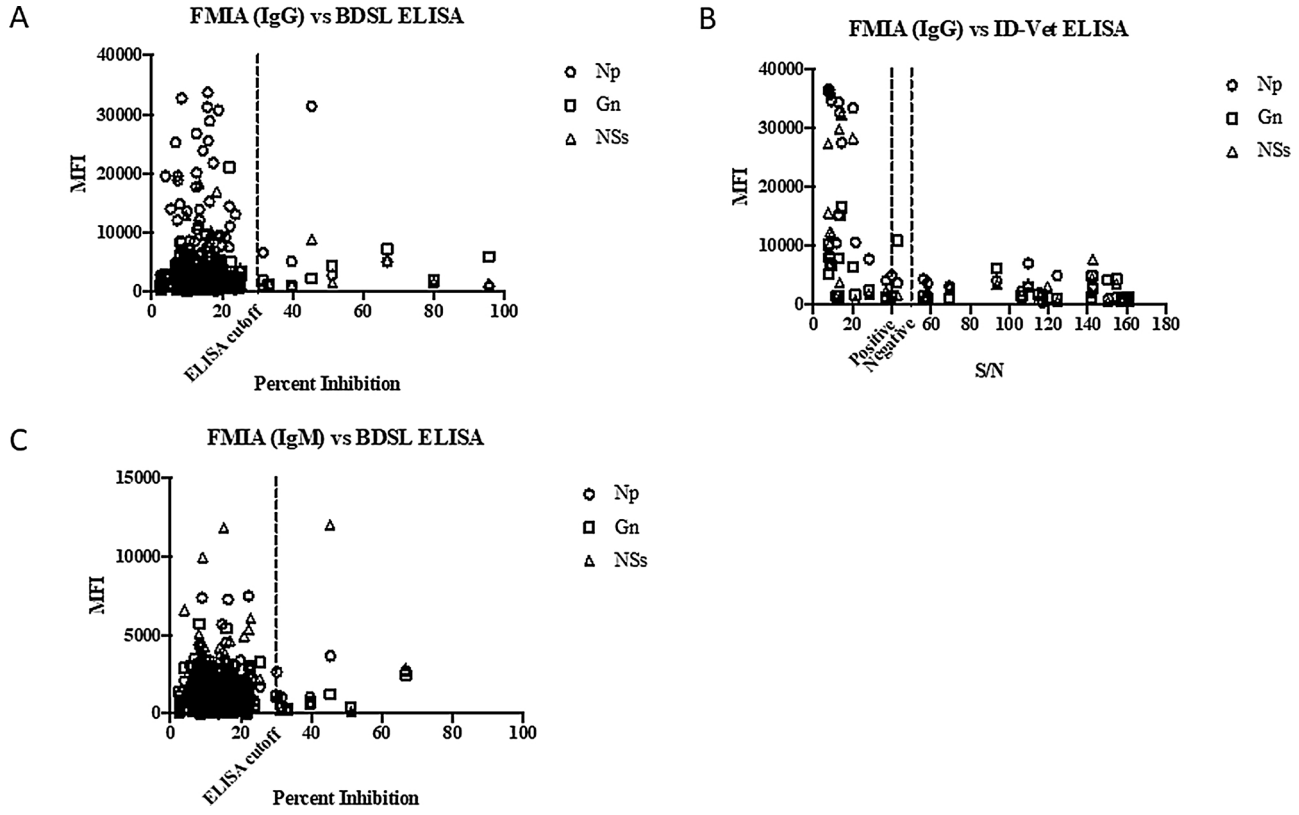

Fig. 1. Comparison of RVFV ELISAs to FMIA. Sheep and cattle samples were tested by FMIA and by either BDSL or IDvet ELISA. Detection of antibodies against three of the RVFV FMIA targets are displayed in MFI on y-axis; Np, Gn, and NSs. Detection of antibodies by ELISA are displayed on x-axis; BDSL test in percent inhibition (cutoff > 30 = positive), IDvet test in percent competition (Sample/Negative * 100%) (cutoff ≤ 40 = positive, 40 < S/N ≤ 50 = inconclusive, > 50 = negative). The FMIA test detected either IgM or IgG. The ELISA tests detected both IgM and IgG.

provided by the Ethics and Scientific Review Committee (ESRC) of the Africa Medical and Research Foundation (AMREF) (REF: AMREF-ESRC P65/2013). A portion of samples had been collected during a cross-sectional survey and tested with the BDSL RVF ELISA (BDSL, National Institute for Communicable Diseases, Centre for Emerging and Zoonotic Diseases, Johannesburg, South Africa) as described previously (Bett et al., 2017; Lindahl et al., 2016). Another set of samples had been collected during a longitudinal sampling and tested with the IDvet RVF ELISA (ID Screen® Rift Valley Fever Competition Multispecies ELISA, IDvet, Grabels, France), as described previously (Mbotha et al., 2017). Neither ELISA used can differentiate IgM and IgG antibodies, and both tests were concluded to be sensitive and specific in a European ring-trial (Kortekaas et al., 2013).

2.2. Coupling of recombinant proteins to microspheres

Recombinant RVFV proteins were produced as previously described (Ragan et al., 2018). Target analytes included RVFV nucleocapsid Np, glycoprotein Gn, and non-structural proteins NSs and NSm. In addition, non-RVFV proteins, porcine circovirus 2 (PCV2) and green-fluorescent protein (GFP), were used as negative control beads to account for non-specific binding of antibodies to the recombinant proteins. A blank bead set with no coupled antigen was used to account for non-specific binding to the beads.

2.3. Luminex assay

Assays were prepared as previously described (Ragan et al., 2018). For antibody detection in sheep sera, samples were diluted 1:400 in assay buffer (1% fish gelatin in PBS with 0.05% Tween-20 and 0.05% sodium azide, pH 7.4). IgG antibodies were detected using secondary rabbit anti-sheep biotinylated IgG (Jackson Immunoresearch Laboratories, West Grove, PA) diluted to 1 μg/mL followed by a fluorescent conjugate streptavidin-phycoerythrin (SAPE) (Moss, Inc, Pasadena, MD) diluted to 1 μg/mL. IgM antibodies were detected with secondary rabbit anti-sheep biotinylated IgM (MyBioSource, San Diego, CA) diluted to 1 μg/mL followed by the fluorescent conjugate diluted to 2 μg/mL.

For antibody detection in cattle sera, samples were diluted 1:200 in assay buffer. IgG antibodies were detected using secondary goat anti-bovine biotinylated IgG (Jackson Immunoresearch Laboratories, West Grove, PA) diluted to 1 μg/mL followed by the fluorescent conjugate diluted to 1 μg/mL. All samples were tested in duplicate.

Plates were analyzed on the Luminex MAGPIX® System using xPONENT version 4.2 software (Luminex Corporation, Austin, TX). The analysis of fluorescence was set to measure 100 beads per bead set per sample. All results were recorded as median fluorescence intensity (MFI). Background signal detected from the blank well containing only beads and assay buffer was subtracted from each sample well. The average MFI was calculated for each duplicate sample and then the mean MFI of the negative control bead set was subtracted. Assay MFI cutoffs for sheep samples were: 2500 for Np target, 6200 for Gn target. For cattle samples: 2900 for Np target, 9400 for Gn target.

2.4. Statistical analysis

Comparisons between previous ELISA results and the results by FMIA were done descriptively. Analysis was done using Graph Pad Prism software (Version 7.0) (Graphpad Software, Inc, La Jolla, CA). Pearson's correlation coefficient was calculated to compare ELISA results to the FMIA antigen targets. A *t*-test was used to analyze signal differences between the FMIA targets.

3. Results

Four RVFV antigen targets on the FMIA (Np, Gn, NSs, NSm) were used to detect antibodies in ruminant sera. Antigen targets Np and NSs showed strong MFI signal on the FMIA for positive sera (Fig. 1). The Gn target also showed good MFI signal although not as strong as Np and NSs (*p-value* < *0.05*). NSm had high background signal (*data not shown*) and was excluded from further analysis. When detected IgG antibodies by FMIA were compared to samples tested by the BDSL ELISA (Fig. 1A), there was low MFI signal for the NSs and Gn but high signal for Np for samples below the ELISA cutoff (negative samples). Interestingly, samples above the BDSL ELISA cutoff (positive samples) had a trend towards a lower MFI for the Np target. When the FMIA was compared to the IDvet ELISA, the MFI signal was overall higher for all three targets with samples below the IDvet cutoff (positive samples) and overall lower signal above the cutoff (negative samples) (Fig. 1B). The correlation between the NSs and Gn targets to the IDvet ELISA was mild at $R^2 = 0.24$ but stronger with the Np target at $R^2 = 0.58$ (*p-value* < *0.05*). When IgM antibodies detected by the FMIA were compared to the BDSL ELISA (Fig. 1C), the overall MFI signals were lower for the targets when compared to IgG detection. As seen previously with the IgG detection, the FMIA had higher MFI signal for samples below the BDSL cutoff. Interestingly, the NSs had the highest MFI signal of the three targets when detecting IgM antibodies compared to the Np target, which had the stronger signal when detecting IgG antibodies. Overall, the detection of IgG antibodies by the FMIA correlated better with the IDvet ELISA than the BDSL ELISA.

Serum from three individual sheep collected longitudinally as described above was also used to compare detection of IgG antibodies by the FMIA and the IDvet ELISA (Fig. 2). There was no rise in MFI signal from any bead sets on the first three sampling dates for the first two sheep. By the fourth sampling date, there was a rise in signal for all three targets. The Np target continued to rise in MFI signal on the fifth sampling date while the other two targets decreased in signal over time. The third sheep had MFI signals rising only for Np and Gn, but the Gn MFI fell under the thresholds, which is consistent with the ELISA results that shifted between positive and borderline. Although not statistically significant, the Np target had the strongest MFI, then NSs, then Gn. Most samples were positive by IDvet ELISA on the fifth sampling days and correlated with the rise of MFI signal on the FMIA panel (*p-value* < *0.05*). Therefore, all three targets of the FMIA were able to detect a rise in IgG antibodies over time similar to what was observed with the IDvet ELISA.

To evaluate the accuracy of the FMIA, the FMIA Np and Gn targets were used to classify disease status of the serum samples and compared to ELISA results. Table 2 summarizes the results of the FMIA compared to the ELISAs by the Np and Gn targets in singleplex and in multiplex. The cutoff values used for the FMIA assay to detect IgG antibodies came from a previous experimental evaluation of the assay (Ragan et al., 2018). When analyzing by the Np target, the FMIA had several false negative samples but very few false positives. When analyzing only the Gn target, the FMIA showed less false negatives but more false positives. When a sample is considered positive by both Np and Gn, the accuracy of the test was similar to what was observed with the Gn target alone. With the IDvet, two samples tested as borderline. Both these samples were positive by Np but only one of the samples were positive by Gn (*data not shown*). Compared to the ELISAs the Np target was more sensitive while the Gn target was more specific.

To further analyze the FMIA accuracy, the results of the Np and Gn targets were compared to the two ELISA tests separately. For the IDvet, all positive samples were correctly identified by the Np target and all negative samples were correctly identified by the Gn target. For the BDSL, there were more false positive samples identified by the Np target compared to when both ELISAs were evaluated together. The specificity of the Gn target remained unchanged when compared to results of both ELISAs together. When Np and Gn targets were used in multiplex, all positive and negative samples were correctly identified as determined by the IDvet (Table 3). However, more false positives were reported when the FMIA multiplex targets were compared to the BDSL. Therefore, the FMIA assay accuracy was more similar to the IDvet ELISA than the BDSL ELISA.

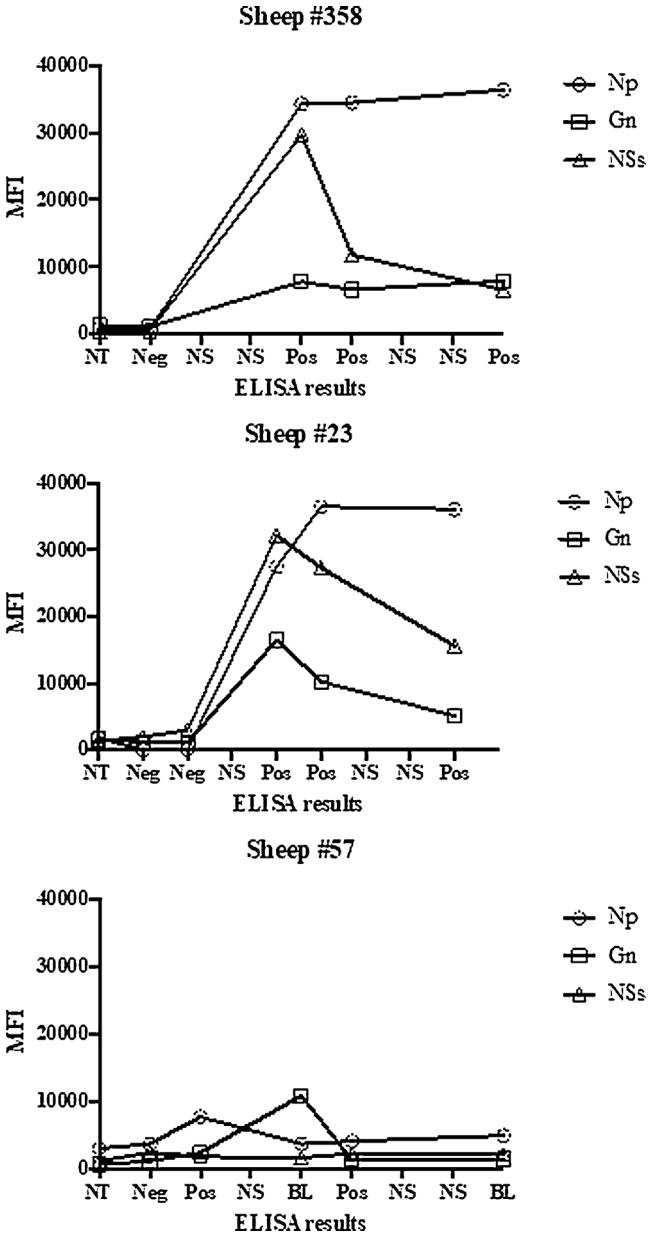

Fig. 2. The results of longitudinal sampled sheep with the ELISA results of the monthly samples on the x-axis (starting September 2014), and the FMIA results on the y-axis. NT: Not tested in the ELISA. NS: Not sampled that month. BL: Borderline result with IDvet ELISA.

4. Discussion

Serological testing is a valuable tool for demonstrating freedom of disease and for surveillance of RVFV globally. The use of bead-based multiplexing platforms allows for simultaneous detection of multiple RVFV targets in one sample. This in turn improves accuracy, reduces sample usage, and allows for high through-put testing. A fluorescence microsphere immunoassay for RVFV detection was previously developed and evaluated with experimental ruminant sera, including evaluation with virus neutralization test, and showed good correlation, also

Table 2
Summary of FMIA and ELISA results for detecting antibodies against RVFV. Sheep and cattle sera samples were tested by BDSL (bovine and ovine sera) or IDvet ELISA (ovine sera). All samples were also tested by FMIA for the detection of IgG antibodies. ELISA results were compared to FMIA against bead targets Np and Gn in singleplex, as well as, Np with Gn in multiplex.

By Np	ELISA Positive	ELISA Negative	Total
FMIA Positive	19	80	99
FMIA Negative	3	95	98
Total	22	175	197
By Gn	ELISA Positive	ELISA Negative	Total
FMIA Positive	10	6	16
FMIA Negative	12	169	181
Total	22	175	197
By Np and Gn	ELISA Positive	ELISA Negative	Total
FMIA Positive	11	2	13
FMIA Negative	12	172	184
Total	23	174	197

Table 3
Summary of FMIA and ELISA by ELISA test. Sheep and cattle samples were tested by FMIA for the detection of IgG antibodies. Samples were classified positive if MFI signal was above cutoff for both Np and Gn bead targets. Results were then compared to the IDvet and the BDSL ELISA for the detection of antibodies against RVFV.

IDvet	ELISA Positive	ELISA Negative	Total
FMIA Positive	9	0	9
FMIA Negative	0	10	10
Total	9	10	19
BDSL	ELISA Positive	ELISA Negative	Total
FMIA Positive	1	2	3
FMIA Negative	6	154	160
Total	7	156	163

at early time points (Ragan et al., 2018). However, it is important that a test is not only evaluated under experimental circumstances but also in field conditions where animals may have been exposed to multiple diseases and different RVFV strains. This study evaluated the FMIA on animal sera from a RVF-endemic area and compared the results to two commercially-available competitive ELISAs.

Four RVFV antigen targets (Np, Gn, NSs, NSm) were evaluated by the FMIA using sheep and cattle sera. Targets Np, NSs, and Gn were used in a longitudinal study while only Np and Gn were used to compare accuracy of the FMIA to ELISAs. Np and Gn have previously shown to be strong targets in detecting IgG and IgM antibodies in ruminant sera (Besselaar and Blackburn, 1991; Bird et al., 2008; Faburay et al., 2013; Fernandez et al., 2012; Jäckel et al., 2013; McElroy et al., 2009; Nishiyama et al., 2016; Paweska et al., 2008, 2007; Ragan et al., 2018). The use of field sera samples also showed strong signals for Np and Gn, demonstrating the immunogenicity of these two targets. NSs also provided a strong signal here; however, it was less sensitive than Np for detecting IgG antibodies as seen with NSs-based ELISAs (McElroy et al., 2009). Interestingly, when detecting IgM antibodies the NSs target had a stronger MFI than Np, which contradicts what was previously seen when a bead-based immunoassay tested experimentally vaccinated sheep sera for IgM antibodies(Hossain et al., 2016). This may be due to the variability in antibody response seen against NSs from animal to animal (Fernandez et al., 2012). With promising results from the NSs target with naturally infected animals as seen in this study, further validation of the target is needed for the FMIA panel. Lastly, the NSm antigen showed high background signal for both negative and positive sera. The target was not used for comparison to ELISAs. Additional optimization of the NSm antigen is needed for use in the FMIA against experimental and field samples. Overall, RVFV Np and Gn are strong immunogenic targets for the FMIA that can be applied in non-endemic, but more importantly, in endemic areas. Additionally, RVFV NSs is a strong candidate target for the FMIA panel.

The FMIA was compared to two competitive ELISAs to determine assay accuracy. Sera from a longitudinal study were previously tested by the IDvet ELISA and offered a comparison to the FMIA by antibody production over time and assay accuracy. The FMIA Np and Gn targets showed to correlate well with the IDvet results for IgG detection. Due to time constraints, comparison of IgM detection of the FMIA to the IDvet ELISA was not performed. The detection of IgM antibodies by the FMIA was tested against results from the cross-sectional study and compared to BDSL ELISA results. Oddly, there was high signal from all FMIA targets for samples that tested negative by BDSL. To test if the high background was due to interference in detecting IgM antibodies, the FMIA was compared to the BDSL ELISA for the detection of IgG antibodies. A similar pattern of high MFI signal for all targets was also noted, indicating the background was not likely from IgM antibody detection. Rather, there is a poor correlation of the FMIA to the BDSL ELISA. This was supported by the lower sensitivity and specificity of the FMIA when compared to the BDSL versus the IDvet. In this preliminary evaluation with limited sample numbers, FMIA results supported the IDvet ELISA results and thus may offer a promising alternative to the IDvet with the advantage of simultaneous testing of several targets. This would allow for rapid screening and confirmation by testing multiple targets against RVFV in one test. Further validation is needed for the FMIA against additional field samples to determine diagnostic accuracy.

In this study, we evaluated field ruminant sera using a multiplexing serological assay for the detection of IgG and IgM antibodies. The results demonstrate comparable accuracy to the commercial IDvet ELISA, yet offering advantages such as versatility, high-throughput testing and cost effectiveness for large sample numbers, since the use of multiple analytes can make the FMIA method more cost-effective than singleplex ELISAs (Ayouba et al., 2017). This assay can be used for routine serological testing and surveillance in endemic areas. More importantly, the FMIA panel can be expanded to include additional pathogen targets to rapidly screen several important livestock diseases such as bluetongue, peste des petits ruminants, Wesselsbron and other abortive pathogens. By doing so would make the methodology more cost efficient and suitable for routine use in low and middle-income countries, where multiple infectious diseases circulate simultaneously.

Funding

This study was part of a project entitled *Dynamic Drivers of Disease in Africa:Ecosystems, livestock/wildlife, health and wellbeing NE-J001570-1* that was jointly funded by the Ecosystem Services for Poverty Alleviation Programme (ESPA) and the CGIAR Research Program Agriculture for Nutrition and Health. The ESPA programme is funded by the Department for International Development (DFID), the Economic and Social Research Council (ESRC) and the Natural Environment Research Council (NERC). The contribution of JL was supported by the Swedish Research Council. This project was supported by the USDA and the C.

Acknowledgements

We would like to thank Dr. Bernard Bett for his support in this study.

References

Adam, I., Karsany, M.S., 2008. Case report: Rift Valley Fever with vertical transmission in a pregnant Sudanese woman. J. Med. Virol. 80https://doi.org/10.1002/jmv.21132. 929–929.

Anyamba, A., Linthicum, K.J., Tucker, C.J., 2001. Climate-disease connections: Rift Valley fever in Kenya. Cad. Saude Publica 17, S133–S140. https://doi.org/10.1590/S0102-311X2001000700022.

Anyangu, A.S., Gould, L.H., Sharif, S.K., Nguku, P.M., Omolo, J.O., Mutonga, D., Rao, C.Y., Lederman, E.R., Schnabel, D., Paweska, J.T., Katz, M., Hightower, A., Njenga,

M.K., Feikin, D.R., Breiman, R.F., 2010. Risk factors for severe Rift Valley fever infection in Kenya, 2007. Am. J. Trop. Med. Hyg. 83, 14–21. https://doi.org/10.4269/ajtmh.2010.09-0293.

Ayouba, A., Touré, A., Butel, C., Keita, A.K., Binetruy, F., Sow, M.S., Foulongne, V., Delaporte, E., Peeters, M., Group, for the, P.S., 2017. Development of a sensitive and specific serological assay based on luminex technology for detection of antibodies to Zaire Ebola virus. J. Clin. Microbiol. 55, 165–176. https://doi.org/10.1128/JCM.01979-16.

Balasuriya, U.B.R., Shi, P.-Y., Wong, S.J., Demarest, V.L., Gardner, I.A., Hullinger, P.J., Ferraro, G.L., Boone, J.D., Cino, C.L., De, Glaser, A.L., Renshaw, R.W., Ledizet, M., Koski, R.A., MacLachlan, N.J., 2006. Detection of antibodies to West Nile Virus in equine sera using microsphere immunoassay. J. Vet. Diagn. Invest. 18, 392–395. https://doi.org/10.1177/104063870601800413.

Besselaar, T.G., Blackburn, N.K., 1991. Topological mapping of antigenic sites on the Rift Valley fever virus envelope glycoproteins using monoclonal antibodies. Arch. Virol. 121, 111–124.

Bett, B., Said, M., Sang, R., Bukachi, S., Lindahl, J., Wanyoike, S., Ontiri, E., Njeru, I., Karanja, J., Wanyoike, F., Mbotha, D., Grace, D., 2015. Land use, biodiversity changes and the risk of zoonotic diseases: findings from a cross-sectional study in tana River County, Kenya. Presentation at the 49th Kenya Veterinary Association (KVA) Annual Scientific Conference 22–25 April 2015.

Bett, B.K., Said, M.Y.M.Y., Sang, R., Bukachi, S., Wanyoike, S., Kifugo, S.C.S.C., Otieno, F., Ontiri, E., Njeru, I., Lindahl, J., Grace, D., 2017. Effects of flood irrigation on the risk of selected zoonotic pathogens in an arid and semi-arid area in the eastern Kenya. PLoS One 12, 1–15. https://doi.org/10.1371/journal.pone.0172626.

Bird, B.H., Albariño, C.G., Hartman, A.L., Erickson, B.R., Ksiazek, T.G., Nichol, S.T., 2008. Rift valley fever virus lacking the NSs and NSm genes is highly attenuated, confers protective immunity from virulent virus challenge, and allows for differential identification of infected and vaccinated animals. J. Virol. 73, 1138–1145. https://doi.org/10.1128/jvi.02501-07.

Chevalier, V., Pépin, M., Plée, L., Lancelot, R., 2010. Rift Valley fever–a threat for Europe? Euro Surveill. Bull. Eur. sur les Mal. Transm. Eur. Commun. Dis. Bull. 15.

Christopher-Hennings, J., Araujo, K.P.C., Souza, C.J.H., Fang, Y., Lawson, S., Nelson, E.A., Clement, T., Dunn, M., Lunney, J.K., 2013. Opportunities for bead-based multiplex assays in veterinary diagnostic laboratories. J. Vet. Diagn. Invest. 25, 671–691. https://doi.org/10.1177/1040638713507256.

Clavijo, A., Hole, K., Li, M., Collignon, B., 2006. Simultaneous detection of antibodies to foot-and-mouth disease non-structural proteins 3ABC, 3D, 3A and 3B by a multiplexed Luminex assay to differentiate infected from vaccinated cattle. Vaccine 24, 1693–1704. https://doi.org/10.1016/J.VACCINE.2005.09.057.

Daubney, R., Hudson, J.R., Garnham, P.C., 1931. Enzootic hepatitis or rift valley fever. An undescribed virus disease of sheep cattle and man from East Africa. J. Pathol. Bacteriol. 34, 545–579. https://doi.org/10.1002/path.1700340418.

Davies, F.G., Martin, V., Nations, F., A.O. of the U, 2003. Recognizing Rift Valley Fever. Food & Agriculture Org.

Elliott, R.M., 1997. Emerging viruses: the Bunyaviridae. Mol. Med. 3.

Faburay, B., Wilson, W., McVey, D.S., Drolet, B.S., Weingartl, H., Madden, D., Young, A., Ma, W., Richt, J.A., 2013. Rift Valley fever virus structural and nonstructural proteins: recombinant protein expression and immunoreactivity against antisera from sheep. Vector Borne Zoonotic Dis. 13, 619–629. https://doi.org/10.1089/vbz.2012.1285.

Fafetine, J.M., Tijhaar, E., Paweska, J.T., Neves, L.C.B.G., Hendriks, J., Swanepoel, R., Coetzer, J.A.W., Egberink, H.F., Rutten, V.P.M.G., 2007. Cloning and expression of Rift Valley fever virus nucleocapsid (N) protein and evaluation of a N-protein based indirect ELISA for the detection of specific IgG and IgM antibodies in domestic ruminants. Vet. Microbiol. 121, 29–38. https://doi.org/10.1016/J.VETMIC.2006.11.008.

Fernandez, J.-C., Billecocq, A., Durand, J.P., Cêtre-Sossah, C., Cardinale, E., Marianneau, P., Pépin, M., Tordo, N., Bouloy, M., 2012. The nonstructural protein NSs induces a variable antibody response in domestic ruminants naturally infected with Rift Valley fever virus. Clin. Vaccine Immunol. 19, 5–10. https://doi.org/10.1128/CVI.05420-11.

Findlay, G.M., 1932. Rift valley fever or enzootic hepatitis. Trans. R. Soc. Trop. Med. Hyg. 25https://doi.org/10.1016/S0035-9203(32)90042-X. 229-IN11.

Hartman, A.L., Powell, D.S., Bethel, L.M., Caroline, A.L., Schmid, R.J., Oury, T., Reed, D.S., 2014. Aerosolized rift valley fever virus causes fatal encephalitis in african green monkeys and common marmosets. J. Virol. 88, 2235–2245. https://doi.org/10.1128/JVI.02341-13.

Hossain, M.M., Wilson, W.C., Faburay, B., Richt, J., McVey, D.S., Rowland, R.R., 2016. Multiplex detection of IgG and IgM to rift valley fever virus nucleoprotein, nonstructural proteins, and glycoprotein in ovine and bovine. Vector-Borne Zoonotic Dis. 16, 550–557. https://doi.org/10.1089/vbz.2014.1721.

Jäckel, S., Eiden, M., Balkema-Buschmann, A., Ziller, M., Jansen van Vuren, P., Paweska, J.T., Groschup, M.H., 2013. A novel indirect ELISA based on glycoprotein Gn for the detection of IgG antibodies against Rift Valley fever virus in small ruminants. Res. Vet. Sci. 95, 725–730. https://doi.org/10.1016/J.RVSC.2013.04.015.

Jeong, Y.E., Jeon, M.J., Cho, J.E., Han, M.G., Choi, H.J., Shin, M.Y., Park, H.J., Kim, W., Moon, B.C., Park, J.-S., Park, B., Ju, Y.R., 2011. Development and field evaluation of a nested RT-PCR kit for detecting Japanese encephalitis virus in mosquitoes. J. Virol. Methods 171, 248–252. https://doi.org/10.1016/j.jviromet.2010.11.009.

Jost, C.C., Nzietchueng, S., Kihu, S., Bett, B., Njogu, G., Swai, E.S., Mariner, J.C., 2010. Epidemiological assessment of the Rift Valley fever outbreak in Kenya and Tanzania in 2006 and 2007. Am. J. Trop. Med. Hyg. 83, 65–72. https://doi.org/10.4269/ajtmh.2010.09-0290.

Kim, H.-J., Nah, J.-J., Moon, J.-S., Ko, Y.-J., Yoo, H.-S., Kweon, C.-H., 2012. Competitive ELISA for the detection of antibodies to Rift Valley fever virus in goats and cattle. J. Vet. Med. Sci. 74, 321–327.

Kortekaas, J., Kant, J., Vloet, R., Cêtre-Sossah, C., Marianneau, P., Lacote, S., Banyard, A.C., Jeffries, C., Eiden, M., Groschup, M., Jäckel, S., Hevia, E., Brun, A., 2013. European ring trial to evaluate ELISAs for the diagnosis of infection with Rift Valley fever virus. J. Virol. Methods 187, 177–181. https://doi.org/10.1016/j.jviromet.2012.09.016.

Lindahl, J., Bett, B., Robinson, T., Grace, D., 2016. Rift Valley fever and the changing environment: a case study in East Africa. In: Bouzid, M. (Ed.), Examining the Role of Environmental Change on Emerging Infectious Diseases and Pandemics. IGI Global, Hershey, PA, pp. 178–204. https://doi.org/10.4018/978-1-5225-0553-2. ch008.

Linthicum, K.J., Davies, F.G., Kairo, A., Bailey, C.L., 1985. Rift Valley fever virus (family Bunyaviridae, genus Phlebovirus). Isolations from Diptera collected during an inter-epizootic period in Kenya. J. Hyg. (Lond.) 95, 197–209. https://doi.org/10.1017/S0022172400062434.

Logan, T.M., Linthicum, K.J., Davies, F.G., Binepal, Y.S., Roberts, C.R., 1991. Isolation of Rift Valley fever virus from mosquitoes (Diptera: Culicidae) collected during an outbreak in domestic animals in Kenya. J. Med. Entomol. 28, 293–295.

Madani, T.A., Al-Mazrou, Y.Y., Al-Jeffri, M.H., Mishkhas, A.A., Al-Rabeah, A.M., Turkistani, A.M., Al-Sayed, M.O., Abodahish, A.A., Khan, A.S., Ksiazek, T.G., Shobokshi, O., 2003. Rift Valley fever epidemic in Saudi Arabia: epidemiological, clinical, and laboratory characteristics. Clin. Infect. Dis. 37, 1084–1092. https://doi.org/10.1086/378747.

Mansfield, K.L., Banyard, A.C., McElhinney, L., Johnson, N., Horton, D.L., Hernández-Triana, L.M., Fooks, A.R., 2015. Rift Valley fever virus: a review of diagnosis and vaccination, and implications for emergence in Europe. Vaccine 33, 5520–5531. https://doi.org/10.1016/J.VACCINE.2015.08.020.

Mbotha, D., Bett, B., Kairu-Wanyoike, S., Grace, D., Kihara, A., Wainaina, M., Hoppenheit, A., Clausen, P.-H., Lindahl, J., 2017. Inter-epidemic Rift Valley fever virus seroconversions in an irrigation scheme in Bura, south-east Kenya. Transbound. Emerg. Dis. https://doi.org/10.1111/tbed.12674.

McElroy, A.K., Albariño, C.G., Nichol, S.T., 2009. Development of a RVFV ELISA that can distinguish infected from vaccinated animals. Virol. J. 6 (125). https://doi.org/10.1186/1743-422X-6-125.

Métras, R., Collins, L.M., White, R.G., Alonso, S., Chevalier, V., Thuranira-McKeever, C., Pfeiffer, D.U., 2011. Rift Valley fever epidemiology, surveillance, and control: what have models contributed? Vector Borne Zoonotic Dis. 11, 761–771. https://doi.org/10.1089/vbz.2010.0200.

Mohamed, M., Mosha, F., Mghamba, J., Zaki, S.R., Shieh, W.-J., Paweska, J., Omulo, S., Gikundi, S., Mmbuji, P., Bloland, P., Zeidner, N., Kalinga, R., Breiman, R.F., Njenga, M.K., 2010. Epidemiologic and clinical aspects of a Rift Valley fever outbreak in humans in Tanzania, 2007. Am. J. Trop. Med. Hyg. 83, 22–27. https://doi.org/10.4269/ajtmh.2010.09-0318.

Moutailler, S., Krida, G., Schaffner, F., Vazeille, M., Failloux, A.-B., 2008. Potential vectors of Rift Valley fever virus in the Mediterranean region. Vector Borne Zoonotic Dis. 8, 749–753. https://doi.org/10.1089/vbz.2008.0009.

Nicholas, D.E., Jacobsen, K.H., Waters, N.M., 2014. Risk factors associated with human Rift Valley fever infection: systematic review and meta-analysis. Trop. Med. Int. Health 19, 1420–1429. https://doi.org/10.1111/tmi.12385.

Nishiyama, S., Slack, O.A.L., Lokugamage, N., Hill, T.E., Juelich, T.L., Zhang, L., Smith, J.K., Perez, D., Gong, B., Freiberg, A.N., Ikegami, T., 2016. Attenuation of pathogenic Rift Valley fever virus strain through the chimeric S-segment encoding sandfly fever phlebovirus NSs or a dominant-negative PKR. Virulence 7, 871–881. https://doi.org/10.1080/21505594.2016.1195528.

OIE, 2014. OIE Terrestrial Manual. Rift Valley Fever (Infection With Rift Valley Fever Virus). Paris.OIE Terrestrial Manual. Rift Valley Fever (Infection With Rift Valley Fever Virus). Paris.

Paweska, J.T., Jansen van Vuren, P., Swanepoel, R., 2007. Validation of an indirect ELISA based on a recombinant nucleocapsid protein of Rift Valley fever virus for the detection of IgG antibody in humans. J. Virol. Methods 146, 119–124. https://doi.org/10.1016/J.JVIROMET.2007.06.006.

Paweska, J.T., van Vuren, P.J., Kemp, A., Buss, P., Bengis, R.G., Gakuya, F., Breiman, R.F., Njenga, M.K., Swanepoel, R., 2008. Recombinant nucleocapsid-based ELISA for detection of IgG antibody to Rift Valley fever virus in African buffalo. Vet. Microbiol. 127, 21–28. https://doi.org/10.1016/J.VETMIC.2007.07.031.

Pepin, M., Bouloy, M., Bird, B.H., Kemp, A., Paweska, J., 2010. Rift Valley fever virus (Bunyaviridae: Phlebovirus): an update on pathogenesis, molecular epidemiology, vectors, diagnostics and prevention. Vet. Res. 41, 61. https://doi.org/10.1051/vetres/2010033.

Ragan, I.K., Davis, A.S., McVey, D.S., Richt, J.A., Rowland, R.R., Wilson, W.C., 2018. Evaluation of fluorescence microsphere immunoassay for detection of antibodies to Rift Valley fever virus nucleocapsid protein and Glycoproteins. J. Clin. Microbiol. 56https://doi.org/10.1128/JCM.01626-17. JCM.01626-17.

Rich, K.M., Wanyoike, F., 2010. An assessment of the regional and national socio-economic impacts of the 2007 Rift Valley fever outbreak in Kenya. Am. J. Trop. Med. Hyg. 83, 52–57. https://doi.org/10.4269/ajtmh.2010.09-0291.

Sang, R., Lutomiah, J., Said, M., Makio, A., Koka, H., Koskei, E., Nyunja, A., Owaka, S., Matoke-Muhia, D., Bukachi, S., Lindahl, J., Grace, D., Bett, B., 2016. Effects of irrigation and rainfall on the population dynamics of rift valley fever and other arbovirus mosquito vectors in the Epidemic-Prone Tana river County, Kenya. J. Med. Entomol. 8https://doi.org/10.1093/jme/tjw206. tjw206.

Tourre, Y.M., Lacaux, J.-P., Vignolles, C., Lafaye, M., 2009. Climate impacts on environmental risks evaluated from space: a conceptual approach to the case of Rift Valley Fever in Senegal. Glob. Health Action 2. https://doi.org/10.3402/gha.v2i0.2053.

Turell, M.J., Sardelis, M.R., O'Guinn, M.L., Dohm, D.J., 2002. Potential Vectors of West Nile Virus in North America, in: Japanese Encephalitis and West Nile Viruses. Current

Topics in Microbiology and Immunology, Springer-Verlag Berlin, Berlin, pp. 241–252.

Turell, M.J., Dohm, D.J., Mores, C.N., Terracina, L., Wallette, D.L., Hribar, L.J., Pecor, J.E., Blow, J.A., 2008. Potential for North American mosquitoes to transmit rift valley fever virus 1. J. Am. Mosq. Control Assoc. 24, 502–507. https://doi.org/10.2987/08-5791.1.

Walter, C.T., Barr, J.N., 2011. Recent advances in the molecular and cellular biology of bunyaviruses. J. Gen. Virol. 92, 2467–2484. https://doi.org/10.1099/vir.0.035105-0.

Watson, D.S., Reddy, S.M., Brahmakshatriya, V., Lupiani, B., 2009. A multiplexed immunoassay for detection of antibodies against avian influenza virus. J. Immunol. Methods 340, 123–131. https://doi.org/10.1016/J.JIM.2008.10.007.

Wilson, W.C., Kim, I.J., Trujillo, J.D., Sunwoo, S.Y., Noronha, L.E., Urbaniak, K., McVey, D.S., Drolet, B.S., Morozov, I., Faburay, B., Schirtzinger, E.E., Koopman, T., Indran, S.V., Balaraman, V., Richt, J.A., 2018. Susceptibility of white-tailed deer to rift valley fever virus. Emerg. Infect. Dis. 24, 1717–1719. https://doi.org/10.3201/eid2409.180265.

Woods, C.W., Karpati, A.M., Grein, T., McCarthy, N., Gaturuku, P., Muchiri, E., Dunster, L., Henderson, A., Khan, A.S., Swanepoel, R., Bonmarin, I., Martin, L., Mann, P., Smoak, B.L., Ryan, M., Ksiazek, T.G., Arthur, R.R., Ndikuyeze, A., Agata, N.N., Peters, C.J., The World Health Organization Hemorrhagic Fever Task, Force, 2002. An outbreak of Rift Valley fever in northeastern Kenya, 1997-98. Emerg. Infect. Dis. 8, 138–144.