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Diagnosis of a Suspected Rift Valley Fever Outbreak Using Capture IgM ELISA in the Sudan

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MBAR, YAS, MEA and ASM investigated the field outbreak and collected samples required for diagnosis and wrote the final report of the disease and described the disease epidemiology. Authors TMAE, MEA and AIN conducted the laboratory work, whereas, authors MAAA and IHA collected the literature search and performed the statistical analysis. The protocol and the first draft of the manuscript, however, were prepared by authors AMEH and AEK. All authors read and approved the final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

During an unusual heavy rainfall season, in October 2007, a serological diagnosis was conducted using Rift Valley Fever (RVF) IgM Enzyme Linked- Immunosorbent Assay (ELISA) for detection IgM immunoglobulin in suspected outbreak of hemorrhagic fever in The White Nile, Gezira, Sinnar and The Blue Nile States of the Sudan. A total of 323 blood samples were collected from cattle, sheep

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and goats and analysed. The overall percentages of IgM antibodies in the three species of animals in the study areas were; 53.6% for caprine, 48.3% for ovine and 21.3% for bovine. Gizera State showed highest morbidity (50%) followed by The White Nile (24.6%) and then Sinnar and Blue Nile states, (21%). Bovine showed low infection whereas caprine morbidity was high followed by the ovine. The overall positive percentage of all animal examined (cattle, sheep and goats) from the Study areas was 35.6% (125/323) Table.

Keywords: Rift Valley Fever virus; ELISA; IgM immunoglobulin.

1. INTRODUCTION

Rift Valley fever (RVF) virus is an arbovirus in the *Bunyaviridae* family that, from phylogenetic analysis, appears to have first emerged in the mid-19th century and was only identified at the begininning of the 1930s in the Rift Valley region of Kenya [1,2]. The disease is an arthropods borne, viral zoontic that threatens the African continent as well as the rest of the world, [3,1]. It is characterized by storm of abortion in all pregnant animals and high mortality rate in their offspring, [4,5,6]. RVF virus, is a member of the *Bunyaviridae* family, genus *Phlebovirus* [7], it is readily transmitted through a wide range of mosquito genera including *Aedes*, *Anopheles*, *Culex*, *Eretmapoites* and *Mansonia*, and by other vectors including sand flies [8,7,9].

Currently circulating strains of RVFV are descended from an ancestral species that emerged from a natural reservoir in Africa when large-scale cattle and sheep farming were introduced during the 19th century [10]. Viruses descended from multiple lineages persist in that region, through infection of reservoir animals and vertical transmission in mosquitoes, emerging in years of heavy rainfall to cause epizootics and epidemics. Recently, 95 unique sequences sorted into 15 lineages were reported by [11].

Studies have illustrated the ability of RVFV to utilize the dominant mosquito species of a given geographical location [7,9,12,13], which indicates that there is no natural blockade to protect RVF- naive countries from the spread of the virus.

Human acquires infection by RVFV from infected animal tissues or mosquito bites [14]. A wide range of symptoms, from fever to fatal encephalitis, retinitis and hepatitis associated with hemorrhages, [15]. Infection in humans usually causes a self-limiting, acute and febrile illness; however, a small number of cases progress to neurological disorders, partial or complete blindness, hemorrhagic fever, or thrombosis [16].

The most important epidemics/epizootics occurred after periods of unusual heavy rainfall, flooding or construction of dams, that flourishes the mosquito population [17,14]. However, on a number of occasions, viruses from these lineages have been transported outside the enzootic region through the movement of infected animals or mosquitoes, triggering outbreaks in countries such as Egypt, Saudi Arabia, Mauritania and Madagascar, where RVF had not previously been seen [10].

RVF was first identified in 1931 by Daubney *et al*, during an investigation of epizootic among sheep on a farm near Naivasha in the Rift Valley of Kenya. The authors isolated the causative viral agent by inoculating lambs with the serum from a moribund sheep, (cited by 13). Initially, the virus was restricted to the eastern region of Africa, but the disease has now spread to southern and western Africa, as well as outside of the African continent, e.g., Madagascar, Saudi Arabia and Yemen. There is a serious concern that the virus may spread to other areas, such as North America and Europe [2].

Since then; many outbreaks were reported in sub-Saharan and North Africa [18]. During 1997-1998, a major outbreak occurred in Kenya, Somalia and Tanzania, [19,18]. In September 2000, two simultaneous epizootics were confirmed in Saudi Arabia and Yemen, marking the first occurrence of the disease outside the African continent [20].

In the Sudan, an outbreak of RVF was first reported in the year 1973 in Kosti region in The White Nile State and some areas of The Blue Nile State [4,5] and later in the year 1980 [6] and large numbers of domestic ruminants were infected. In the year1976, the disease occurred in Khartoum State at Hillat Kuku farm, Khartoum North [21]. These States are agricultural areas and the climatic event resulted in unusual growth

of vegetation. It also favored the high densities of mosquitoes and other biting flies, (water reserves and dams). Animal presence in the area of study is an as essential element in disease occurrence.

This investigation was carried out during an outbreak of faetal hemorrhagic disease in cattle, sheep and goats in Central Sudan (The White Nile, Gezira, Sinnar and The Blue Nile States) using RVF IgM ELISA test as one of the powerful serological techniques to detect presence of RVF IgM antibodies in sheep, goats and cattle.

2. MATERIALS AND METHODS

2.1 Study Area

The outbreak occurred at Central Sudan and particularly in The White Nile, Gezira, Sinnar and The Blue Nile States (Fig. 1) during heavy rainy season from Jun to October, 2007. These areas where were among the flooded parts of the country. The outbreak was predicted in June, 2007 using satellite measurements, of sea surface temperature, vegetation index and elevated rainfall data, [3].

2.2 Samples Examined

Blood sample were collected from bovine, ovine and caprine using 10 ml plain vaccutainer and sterile needles (19 G; for each animal). The collected blood was allowed to clot for up to 24 hours in the shade; sera were transferred into labeled cryovials with identified flock numbers and the designated geographic locality in the study area. The cryovials were kept in deep freezer at -20°C and transferred to the laboratory in cooled containers with ice bags.

2.3 RVF ELISA Kit

Commercial IgM ELISA kits for detection of RVF IgM antibodies were purchased from the National Institute of Communicable Diseases (NICD), Johannesburg, South Africa.

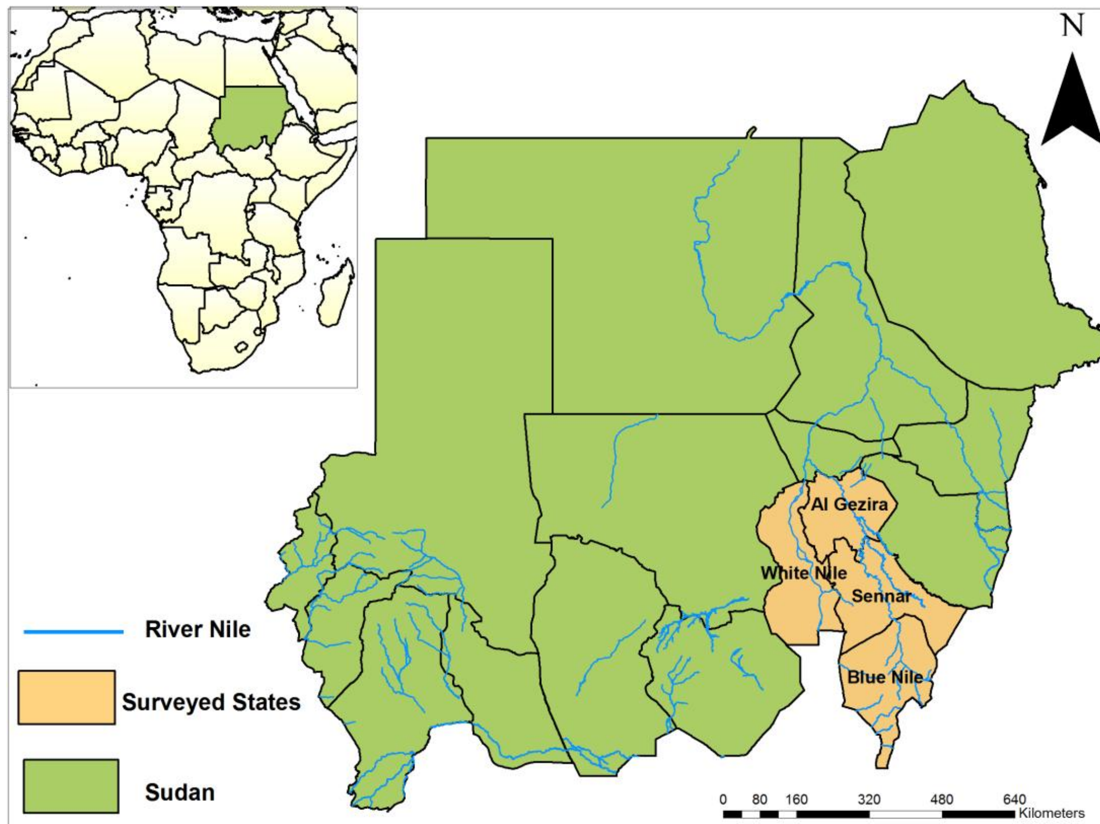

Fig. 1. Illustrates the study area of the Sudan (The White Nile, Gizera, The Blue Nile and Sinnar States).

2.4 Test Procedures

The RVF IgM ELISA was conducted according to the manufacturer's instructions. Plates were coated with rabbit anti–sheep IgM diluted at 1: 500 in PBS, covered with lid, incubated at 4°C overnight. They were washed three times for 15 seconds using 300 µl washing buffer (0.1% Tween 20 in PBS). A volume of 200 µl per well blocking buffer (10% skim milk in PBS) was added and the plates were incubated for one hour in moist chamber at 37°C. Thereafter, they were washed as above. A volume of 100 µl test and control sera diluted at 1: 400 in diluent buffer (2% skim milk in PBS) were added to the wells and then incubated. One hour later, the plates were washed as above. A volume of 100 µl RVFV Ag and control Ag were diluted at 1:400 and added to rows A-D, 1-12, rows E-H, 1-12 respectively. They were incubated for one hour then plates were washed three times. Hundred µl of mouse anti RVFV serum was diluted 1:500 and added to each well. They were incubated for one hour and then, the plates were washed three times. A volume of 100 µl per well anti–mouse IgG HRPO conjugate was diluted at 1:6000 and added, incubated for one hour and then washed 6 times. A volume of 100 µl ABTS substrate was then added and the plates were kept in dark room for thirty minutes at room temperature (25°C). Finally, 100 µl of 1% sodium dodecyl sulphate solution (SDS) was used as stop solution. Optical densities (OD) were read at 405 nm filter.

3. RESULTS

The results showed that 10 (23%) bovine, 38(51%) ovine and 63 (61%) caprine were positive to recent infection of RVF in Gezira State. However, 5 (17%) bovine, 6 (50%) ovine and 4 (18%) caprine were positive to recent infection of RVF in The White Nile State. In Sinnar and The Blue Nile States no caprine sample were tested , but 7 (22%) bovine and 1 (14%) ovine, were positive (Table 1; Fig. 2). In addition, the overall positive percentage of infected cattle, sheep and goats from the four states collectively was 35.6% (125/323) Table 2.

4. DISCUSSION

The increased demand for rapid diagnostic assays worldwide aims to ensure the protection of human and livestock to facilitate the free circulation of animals and animal's products for International trades. ELISA is a more sensitive test for the detection of border line levels of antibody to RVF [22].

Table 1. Results of serum samples of caprine, ovine and bovine tested by RVF IgM Captured ELISA from The White Nile, Gezira, Sinnar and The Blue Nile States of the Sudan.

Location	Animal species									Total tested
	Caprine			Ovine			Bovine			
	Sample tested	+ve	PP*	sample tested	+ve	PP*	sample tested	+ve	PP*	Total
Gezira	103	63	61%	74	38	51%	43	10	23%	220
The White Nile.	22	4	18%	14	7	50%	29	5	17%	65
Sinnar and The Blue Nile.		--		7	1	14%	31	7	22%	38
Total	**125**	**67**		**95**	**36**		**103**	**22**		**323**

**PP: positive percentage for Elisa test.*

Table 2. The overall positive percentage of IgM antibodies detected in sera of all animal examined (bovine, ovine and caprine) from The White Nile, Gezira, Sinnar and The Blue Nile States of the Sudan.

Anim. spp	Total No. Tested	Total No.+ve	PP*
Cattle	103	22	+7
Sheep	95	36	+9
Goats	125	67	+77
Overall Positive	**323**	**125**	**35.6%**

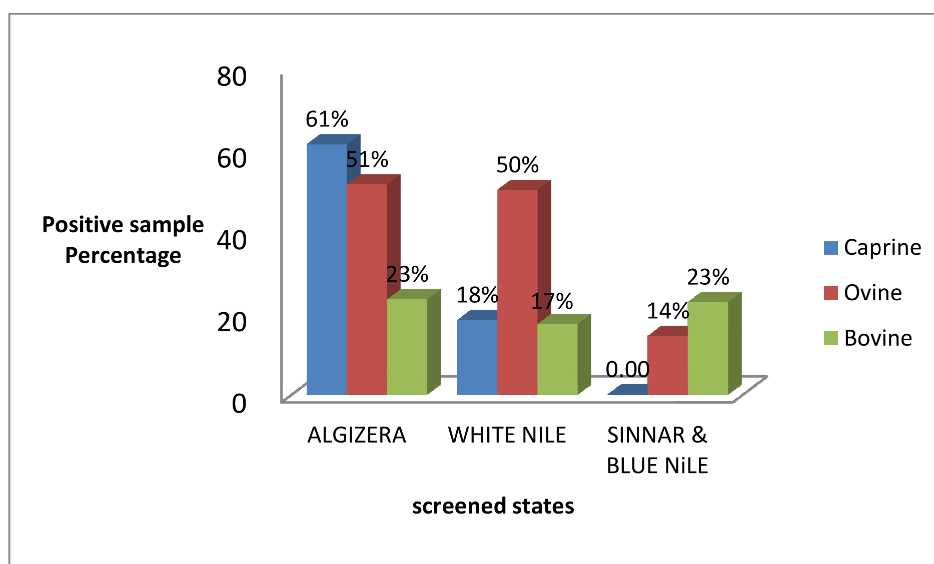

Fig. 2. Number and Positive percentage of cattle, sheep and goats tested in the study Stats.

Rift Valley Fever was previously reported in the Sudan by serological tests that indicated the presence of IgG antibodies to RVFV in animals and human sera, [23,21,4,24,25,26]. Data collected by the aforementioned authors, indicated that RVF was prevalent in subclinical form. The lack or the poor reporting system used for detection of clinical cases did not reflect the real situation in the country. In addition, the indigenous animals might not show the classical clinical signs due to innate resistance, [27]. Therefore, the virus activity may only be detected by occasional human disease or by random isolation from mosquitoes, [27]. Central Sudan was formerly hit by RVF outbreak in 1973 [21].

In this investigation, heavy prolong rainfall and flooding occurred in The White Nile and The Blue Nile, in the year 2007. This was accompanied by a massive abortion in animal species, and the widespread of hemorrhagic disease in human, beside the high prevalence of IgM antibodies positive for RVF obtained, indicated that this was an outbreak of RVF. The disease was initially seen in The White Nile State, then in the Gezira State which was badly affected. The situation in Gezira might be due to its different topography, the abundance of canals and rich grasslands, which might favour the increase of insect population. All these factors might have led to the severe epizootic of the disease in the area.

During this investigation small ruminants were worst affected compared to cattle, and goats were severely affected compared to sheep. On the contrary, this result is completely different from the year 2003 outbreak in Khartoum and River Nile States, where cattle were badly affected. This might be due to the high foreign blood cattle reared in the two States, as well as, the small population of small ruminants in them.

Since no RVF vaccination campaign was launched before in Central Sudan, the presence of IgM antibodies is indicative for active and circulating virus in the investigated area. It is worth to mention that, no virus isolation was carried out and (as recommended by the OIE). Moreover, the classical methods for the detection of antibodies to RVFV included Haem-agglutination-inhibition, Complement Fixation, Indirect Immuno-fluorescence and Virus Neutralization tests [28]. Disadvantages of these techniques include health risk to laboratory personnel [29] and restriction of their use outside RVF endemic areas [30]. Although, the virus neutralization test is regarded as a gold standard, the test is laborious, expensive and requires 5-7 days for completion. It can be performed only in specialized reference laboratories where standardized stock of live virus and tissue culture are available. [22].

The feasibility of the IgM ELISA to detect antibody to RVF has been reported [22,28,31]. The test was recommended for diagnosis of RVF

worldwide [28,31,32] and it proofed to be sensitive, specific, cheap and easy to be performed. The sensitivity of IgM Capture ELISA was 100%, and the specificity ranged from 99.29% to 100% [29].

Delay in diagnosis associated with conventional virus isolation and identification techniques may represent a significant problem for regulatory health-care authorities faced with an epidemic of RVF, especially outside its traditional geographical territories, [19]. However, traditional and molecular procedures for diagnosis of RVF may be beyond the resources and capabilities of many laboratories, particularly in developing countries, [22].

It is worth to mention that RVF surveillance, disasters, outbreak investigations management, delivery of healthcare; systems-related approaches require flexible approach. In the past, the Ministry of Health, the Ministry of Agriculture and Animal Resources, other government organizations, academic institutions and NGOs had separate roles with little overlap. Despite limited resources, the concept of One Health approach is acceptable approach that intended to develop collaborative leaders committed to improving health equity and social justice by addressing health disparities that impact on efficiency by promoting shared resources and collaboration among those working at the animal (wildlife, livestock and companion animals), ecosystem and human health interface [33].

5. CONCLUSION

This investigation showed that the overall percentages positivity of IgM antibodies in the three animal species examined in the study areas were; (53.6%) for caprine, (48.3%) for ovine and (21.3%) for bovine. Gizera State showed highest morbidity, (50%) rate compared to The White Nile (24.6%) and Sinnar and Blue Nile States, (21%). Presence of IgM antibodies is indicative of active and circulating virus in study area, but no virus isolation was conducted.

It can be concluded that IgM ELISA was recommended before by other investigators and was tested in the present investigation. It proofed to be sensitive, specific, cheap and easy to be performed. It can be used for diagnosis of recent acute infection of RVF worldwide. and for the monitoring of immune response in vaccinated animals as required by the OIE guide lines. It is highly accurate, safe test and has the potentiality to replace conventional diagnostic techniques which pose biohazard risks that limit their use outside endemic areas.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Pepin M, Michèle Bouloy M, Brian H, Bird BH, Alan Kemp A, Paweska J. Rift Valley fever virus (*Bunyaviridae: Phlebovirus*): an update on pathogenesis, molecular epidemiology, vectors, diagnostics and prevention. Vet. Res. 2010;41(6):61. DOI: 10.1051/vetres/2010033
2. Terasaki K, Tercero BR, Makino S. Single-cycle replicable Rift Valley fever virus mutants as safe vaccine candidates. Virus Res. 2016;2(216):55-65.
   DOI: 10.1016/j.virusres.2015.05.012
3. FAO. Flood cause damage in parts of several East African countries, Global information and early warning system. Global watch. Food and Agriculture Organization September 2007. Rome; 2007.
4. Eisa M, Obeid HMA, Elsawi ASA. Rift valley fever in the Sudan. I. Results on field investigations of the first epizootic in Kosti District, 1973. Bull. Anim. Hlth. Prod. Afri. 1977b;24:343-347.
5. Eisa M, Obeid HMA. Rift valley fever in the Sudan. II. Isolation and identification of the virus from a recent epizootic in kosti District, 1973.Bull. Anim. Hlth. Prod. Afri. 1977a;24:349-355.
6. Eisa M, Kheirelseed ED, Shommein AM, Meegan GM. An outbreak of Rift valley fever in the Sudan. Transaction of Royal Society of Tropical Medicine and Hygiene. 1980;74(3):417-419.
7. McIntosh BM, Jupp PG, Dos Santos I, Barnard JH. Vector studies on Rift Valley Fver in South Africa. South Afri. Med. J. 1980;58:127-132.
8. Fontenille D, Traore-Lamizana M, Diallo M, Thonnon J, Digoutte J, Zeller H. New vectors of Rift Valley fever in West Africa. Emerg. Infect. Dis. 1998;4:289–93.

9. Moutailler S, Krida G, Schaffner F, Vazeille M, Failloux AB. Potential vectors of Rift Valley Fever Virus in the Mediterranean region. Vect. Born. Zoon. Dis. 2008;8: 749–53.
10. Ikegami, T. Molecular biology and genetic diversity of Rift Valley fever virus. Antiviral Res. 2012;95(3):293–310.
    DOI: 10.1016/j.antiviral.2012.06.001
11. Grobbelaar AA, Weyer J, Leman PA, Kemp A, Paweska JT, Swanepoe R. Molecular Epidemiology of Rift Valley Fever Virus. Emerg. Infect. Dis. 2011; 17(12):2270-2276.
12. Turell MJ, Kay BH. Susceptibility of selected strains of Australian mosquitoes (Diptera: Culicidae) to Rift Valley fever virus. J. Med. Entomol. 1998;35:132–5.
13. Turell MJ, Perkins PV. Transmission of Rift Valley fever virus by the sand fly, Phlebotomus duboscqi (*Diptera: Psychodidae*) Americ. J. Trop. Med. Hygi. 1990;42:185–8.
14. Flick R, Bouloy M. Rift valley fever virus. Curr. Mol. Med. 2005;5:827-834.
15. Liu L, Cristina CP, Celma CC, Roy P. Rift Valley fever virus structural proteins: expression, characterization and assembly of recombinant proteins. Virol. J. 2008; 5:82.
16. Ikegami T, Makino S. The pathogenesis of Rift Valley fever. Viruses. 2011;3(5):493-519.
17. Tara K, Harper TKH. Virology Notes; 2002.
18. FAO. Food and Agriculture Organization Contingency Plan. Rome; 2003.
19. Anderson GW, Saluzo JF, Ksiazek TG, Smith JF, Ennis W, Thureen D, Peters CJ, Digoutte JP. Comparison of *in vitro* and in *vivo* systems for propagation of Rift Valley Fever virus from clinical specimens. Res. Virol. 1989;140:129-138.
20. Anon. Outbreak of Rift Valley Fever Saudi Arabia, August-October. Centers for Disease Control and Prevention. Morbidity and Mortality Weekly Report. 2000;905-908.
21. Eisa M. Preliminary survey of domestic animals of the Sudan for precipitation antibodies to Rift valley fever virus .J. Hygi. 1984;93:629-637.
22. Paweska JT, Burt FJ, Anthony F, Smith SJ, Grobbelaar AA, Croft JE, Ksiazek TG Swanepoel R. IgG-sandwich and IgM-capture enzyme-linked immunosorbent assay for the detection of antibody to Rift Valley fever virus in domestic ruminants. J. Virol. Meth. 2003;113(2):103-112.
23. Findlay GJ, Stephanopoulo GM, Mac Callum F. Presence d'anticorps contre le fievere de la vallee du rift dans le san Africans. Bulletin de la Societe des Pathologie Exotique. 1936;29:987-996.
24. Watts DM, El Tigani A, Botros BA, Salib AW, Olson JG, McCarthy M, Ksiazek TG. Arthropod-borne viral infections associated with a fever outbreak in the northern province of Sudan. J. Trop. Med. Hygi. 1994;97(4):228-230.
25. Kambal SMOA. Prevalence of Rift Valley fever in Shambat, Khartoum North. M.Sc. Thesis. University of Khartoum, Sudan; 1997.
26. Abedel Rahim ME, Ali MHE, Tamador MA.E, Fadol MA. A survey of Rift Valley Fever Virus antibodies in domestic animals in the Sudan.(unpublished data); 2004.
27. FAO. Food and Agriculture Organization News Report. Rome; 2002.
28. Swanepoel R, Struthers JK, Erasmus MJ, Shepherd SP, McGillivaray GM, Erasmus BJ, Barnard BJ. Comparison of techniques for demonstrating antibodies to Rift Valley Fever Virus, J. Hygi. 1986;97:317-329.
29. Paweska JT, Elizabeth M, Patrecia AL, Swanepoel R. An inhibition enzyme-linked immunosorbent assay for the detection of antibody to Rift Valley Fever in humans domestic and wild ruminants. J. Virol. Meth. 2005;127(1):10-15.
30. Barnard BJH, Gerdes GH. Rift Valley fever, in OIE manual of standards for diagnostic tests and vaccines. 4th edn. Office International de Epizooties, Paris, France. 2000;144-152.
31. Tamador MAE. Epidemiology of Rift Valley Fever in Sudan PhD thesis. Faculty of Veterinary Science, University of Khartoum. Sudan; 2007.
32. WHO/FAO Working Group on Rift Valley Fever. Rift Valley Fever: An Emerging

Human and Animal Problem. ITS Publication. Geneva. 1982;63:69.

33. Nyatanyi T, Wilkes M, McDermott H, Nzietchueng S, Gafarasi I, Mudakikwa A, Kinani JF, Rukelibuga J, Omolo J, Mupfasoni D, Kabeja A, Nyamusore J, Nziza J, Hakizimana JL, Kamugisha J, Nkunda R, Kibuuka R, Rugigana E, Farmer P, Cotton P, Binagwaho A. Implementing one health as an integrated approach to health in Rwanda. BMJ Global Health. 2017;2(1):121.

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