OPEN ACCESS

GAVIN PUBLISHERS

Journal of Vaccines, Immunology and Immunopathology

Research Article

Kamal SA. J Vaccines Immunol: JVII-126.
DOI: 10.29011/2575-789X. 000026

Some Virological Studies on Rift Valley Fever Virus in Camels

Samia Ahmed Kamal*

Department of Virology, Animal Health Research Institute, Giza, Egypt

***Corresponding author:** Samia Ahmed Kamal, Department of Virology, Animal Health Research Institute, Dokki, Giza, Egypt. Email: selkabany@yahoo.com

Citation: Kamal SA (2018) Some Virological Studies on Rift Valley Fever Virus in Camels. J Vaccines Immunol: JVII-126. DOI: 10.29011/2575-789X. 000026

Received Date: 09 February, 2018; **Accepted Date:** 16 February, 2018; **Published Date:** 23 February, 2018

Abstract

Background: Rift Valley Fever Virus (RVFV) infected camels plays certain roles in transmitting pathogens to a new land. Egypt imports camels from the African countries. The camels arrive in Egypt by sea and border crossings with the Sudan and Libya. The present study performed on imported, non-vaccinated camels. This research aims to help in protection of camel from diseases.

Materials and Methods: Blood, sera, portal blood and tissue specimens were collected and examined by virology techniques [Agar Gel Precipitation Tests (AGPT), Immune Fluorescence tests (IF), Enzyme Linked Immune Sorbent Assays (ELISA)] and histopathology. SNT (serum neutralization test) was applied.

Results: RVFV antigen was detected in imported camels by AGPT and IF. IF test showed the inclusions and apoptotic bodies were giving special fluorescence that identical to histopathology changes. ELISA and SNT showing the presence of neutralizing antibodies against RVFV.

Conclusions: Camel plays certain roles in transmitting RVFV, but immune system weaken the virus under ordinary conditions. So that, other environmental factors needed to convert the weak virus into its virulence status.

Keywords: Rift Valley Fever Virus; RVFV; RVF in Camels

Introduction

Rift Valley fever (RVF) is a peracute, acute, sub-acute and / or chronic disease of man and animals. RVFV consists of a single serotype of the genus Phlebovirus; family Bunyaviridae, was first isolated from sheep and man in Kenya in 1930 [1]. RVFV has the potential to infect a remarkable array of arthropods vectors, including mosquitoes, and other hematophagous insects [2]. The virus is enveloped, spherical, icosahedral small particles of about 80-120 nm in diameter. The virus has three segments of single-stranded RNA genome. The three segments described as: L (large), M (medium) and S (small), each segment is contained in a separate nucleocapsid within the virion. The S segment is ambisense RNA (has bi-directional coding) [3]. Recent investigation mention that the replication of RVFV genome segments in cell level starts locally at the site of fusion of the virion with the endosome, and within 4-6 hours, it continues, to proceed throughout the cytoplasm. After the replication phase, genome segments are recruited to the Golgi. However, the co-infection with complementing particles may result in productive infection; moreover, that virions containing antigenomic-sense RNPs may also contribute to the RVFV infection cycle [4,5].

In Egypt, RVFV was first transmitted from the Sudan via camels and other ruminants and cause huge outbreaks in 1977, in which camels, sheep, cattle, buffaloes and goats were affected [6]. RVFV isolated from camel sera at Daraw province (Aswan governorate) during the 1977 Egypt epizootics [7]. Also, RVFV was isolated from sera collected from serologically negative clinically healthy 50 camels in Qena and Aswan quarantines in 1978. Isolation of the virus from the blood of apparently healthy camel, denoted that camels may act as a carrier of the virus and can play an active role in its transmission [8]. Camels have long been exported to Egypt from the Sudan for various purposes. An author, demonstrated HI antibodies to RVFV in over 30% of camels sampled at the southern border of Egypt [9]. So that these authors suggested the virus was introduced into Egypt during the 1977-1978 epizootic by camels from the South and the camels have a

role in maintaining RVFV circulation. Camels plays major role in crossing borders and transmitting RVFV to new lands.

However, RVF neutralizing antibodies were detected in camels and widespread infections and abortions in camels were observed during RVF outbreaks in Kenya, Egypt, and Arabian Peninsula. RVF virus was previously isolated from blood samples from healthy, naturally infected camels in Egypt and the Sudan [8,10]. Also, in Mauritania (September-October 2010), an outbreak of Rift Valley fever was reported in the northern Sahelian region, when the camels apparently played a central role in the natural amplification of the virus. The massive infections have followed slaughter of infected camel by a family in which all members were getting infected with RVFV following handling camel meat and exposure to the blood. During this outbreak, two clinical forms were observed in camels: Per-acute form, with sudden death in less than 24 hours; and acute form with fever, various systemic lesions and abortions. When hemorrhagic signs developed in camels, death usually occurred within a few days in manner resembles infections in the most susceptible species [11]. Camel is considered a beautiful creature and does not require much food and drink and help its owners provide them with milk in a difficult environment. However, RVFV infected camels are transmitting pathogens to a new land. The aims of this work were directed to investigate the role of camel in transmitting RVFV to Egypt.

Materials and Methods

Reference sera and reference RVFV antigens, were kindly supplied by research sector holding company for vaccines and sera Egypt (VACSERA). This research was performed in AHRI, Shalatin district quarantine (Red Sea Governorate) and AHRI regional laboratory and VACSERA Egypt.

Animals

Swiss mice (6 days old) were supplied by VACSERA (Egypt), and subjected for isolation of RVFV from the samples which were collected from the imported Camels. Some samples were collected from abattoirs in Cairo, Giza.

Samples for Virological Investigation

Sera, whole blood, swaps were collected from live animals, however, the camel in this study were not vaccinated against RVFV. In addition to samples from hepatic blood that collected from the portal veins. Tissue specimens (liver and kidneys) were collected from the slaughtered camels at abattoirs and butchers. Sera samples were inactivated at 56 C for 30 minutes before being used in the Serum Neutralization Test (SNT) according to Edwin and Nathalie,1979 [12].

Histopathology Studies

Tissue specimens either slaughter for food or died naturally from the same timing and locality of this study were undergone pathological and virological examinations. The tissue specimens were divided into two parts one for virology study that kept in -20 and the other part kept in 10 % formalin solution for subsequent pathological studies [13,14].

Tissue culture Cell lines

VERO cell line was prepared and provided by VACSERA Egypt. The VERO cells were used for isolation, adaptation of RVFV, virus titration as well as Serum Neutralization Test (SNT) [14].

RVFV Strain

RVFV pantropic Menya Strain (Menya /Sheep/258) was kindly provided by applied research sector VACSERA, Egypt. RVFV was of infectivity titer in the order of 7.5_{log} (10) / ml [14].

RVFV titration and determination of infectious dose (ID50)

VERO cell lines were used for determination of virus' infectivity titer. 50% tissue culture infectious dose of a virus (TCID50), was carried out by traditional methods of virus quantification. Viral infectivity titer was evaluated according to the method adopted by Reed and Muench [15].

Trial for RVF Virus Isolation

Mice aged 6 days were inoculated intra-peritoneal by buffy coats and tissue homogenates samples collected from imported camels according to (Findlay and Howard, 1952). Two mice were used for every prepared buffy coat sample and observed daily for 8 days post-inoculation. 0.2 ml from each buffy coat sample was inoculated into mice using i/p injection (2 mice for each sample) and observed daily for day 8 post- inoculation, or appearance of clinical symptoms or death (paralysis and death) [14].

Tissue Culture Inoculation

VERO used for isolation of RVF virus. Each prepared buffy coat sample was inoculated into VERO cell line tissue culture (3 tissue culture tube used for each sample). Detecting the maintenance medium before inoculation. 0.1 ml per each tube from each sample was inoculated into cell culture tube and left for one hour for adsorption. 100 ul maintenance medium containing 2% inactivated bovine serum was added. The tube was incubated at 37 C and observed daily for 5 days post-inoculation for evidence of cytopathic effect [14].

Virus Neutralization Test (VNT), plaque test: Serial dilutions of heat inactivated test serum are prepared in a 96 well plate and are incubated with a set amount (100 $TCID_{50}$) of infectious virus. Following this incubation, virus susceptible cells are added to the virus-serum mixture, and the final virus/serum/cell combination is incubated for a period of 2-3 days. After this incubation period the test is read by examining each well of the plate for the presence of viral infection. Depending on the virus, this may be by direct microscopic examination of the plate for evidence of viral Cyto-

Pathic Effect (CPE) or, if the virus causes little or no CPE, by immune fluorescent staining of wells for the presence of viral antigen in the cell monolayer [14].

Immune Fluorescent Antibody Technique (IF): In our study, camel' buffy coats, impression smears, mice liver tissue sections (that used for isolation of RVFV from the samples), portal blood smears and liver tissue sections of camel were subjected for IF tests. The detection of RVFV antigen in infected and control was applied by direct Immunofluorescence Technique (IF) according to [14]. The control negative, control positive monolayer tissue cultures, and the control tissue sections were kindly provided by VACSERA Egypt.

Agar Gel Precipitation Tests (AGPT): This test was applied on buffy coats, portal blood, tissue homogenates, and the tissue culture isolates. The reference polyclonal antisera against RVFV was kindly provided by VACSERA Egypt. The principle for the AGPT is the migration of soluble antigens and soluble antibodies toward each other through the agar gel matrix. RVFV antigen and the known specific antibodies would complex to form a precipitate which is trapped in the gel matrix and produces a visible line [14].

ELISA: Antibody detection technique, conventional methods: The test performed according to [14].

Results

Clinical Symptoms

A large number of pregnant and suckling camels were seen entering Egypt coming from Africa. The General Authority for Veterinary Services claimed that Egypt does not import female camels nor young camels because they are susceptible to Rift Valley Fever virus. The effect of Rift Valley Fever virus in camels is not easily detected since camels live in the desert. Camels under study were mostly either apparently normal or suffering from emaciation, dehydration, face and neck edema, tick infestations, hemorrhages from nostrils, skin lesions, and some deaths. We noticed that there are large numbers of mosquitoes in both Shalatin and Halaib and the rest of the South. We also noticed that the mosquito's vectors are huge in the Abu Simbel area. We have also noted the absence of mosquito control measures, whether individual or governmental (Figures 1-4).

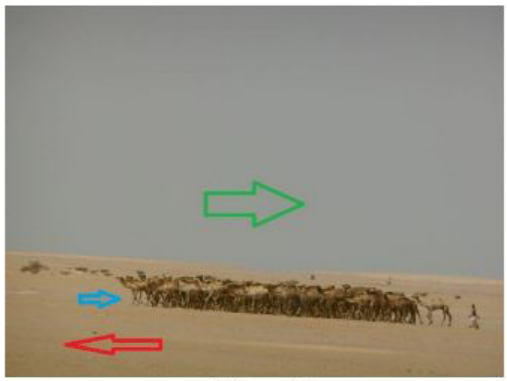


Figure 1

Figure 2

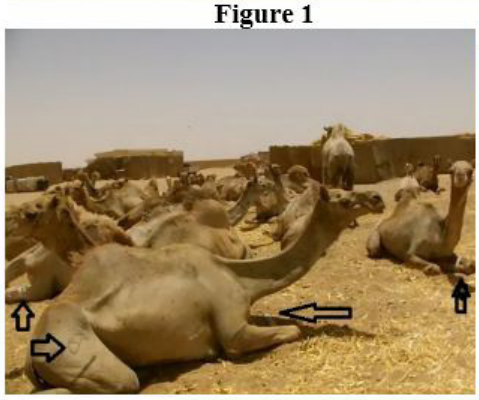


Figure 3

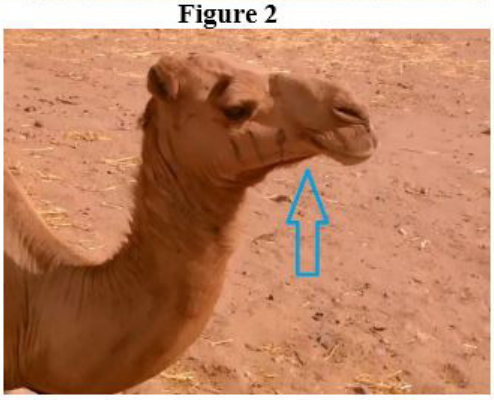


Figure 4

Figures (1-4): Imported camels in Shalatin and Halaib, no. 1 (Dabouka at Abu Ramad) just entering Egypt land coming from the Sudan through Hadriba. No.2 Shalatin market, tourists watching camels. No. 3 and 4 camels in Shalatin market suffering signs of illness.

Agar Gel Precipitation tests (AGPT): (Table 1) showing the results, many cases were positive, buffy coats were the most suitable for this rapid test. Positive reference sera (Ab) against RVFV antigen (Ag) appear as whitish line as an expression of Ag and Ab complex which is trapped in the gel matrix and produces a visible line (Figure 5)**.**

Sample type			Age			Tests				Comments
Sera	Buffy Coat	Tissue, swaps	Less than 1year	1-3 year	Above 3 years	SNT	AGPT	IF	ELISA	
50	50	50		√	√	37	10	10	46+	Apparently normal, Giza
3 Mother and in contact	3 Mother and in contact	1 (liver, aborted fetus)	Aborted fetus		√	1	1	1	3+	Fever, Abortion.
100	100	0			√	70	16	28	78+	Shalatine Quarantine, via the Sudan (African breeds). Samples collected from; Camels showed ill conditions (fatigue, emaciation, congested conjunctiva, lacrimation, diarrhea, skin lesions, ticks, edema)
100	100				√	34	81	20	54+	Great Pilgrim, Slaughter houses, Cairo
		6 (liver)			√		4	4		Butchers, livers showed congestion
20	20				√	20	14	20	13+	Camel Market, imported camels from Madagascar. Samples collected from Camels showed ill conditions (fatigue, emaciation, congested conjunctiva, lacrimation, diarrhea, skin lesions, ticks, edema)

Table 1: Virology results. Imported camels [females and males / adult and young].

Figure 5: Positive AGPT.

ELISA: (Table 1) are showing the positive samples.

Histopathology Results

(Figures 6-21) showing the pathological changes caused by RVFV and most probably as direct and indirect effects of viral infection. However, many shapes of inclusion bodies which indicates that they are viral particles aggregations that proved by identical IF pictures with the histopathological microscopical examinations. The liver showed necrotic foci (necrogranuloma) contains necrotic debris, proliferated Kupffer cells (Figures 6,7), blood monocytes (Figure 10) and lymphocytic infiltration [11,12], hemorrhages (Figures 16,18,19), congested blood vessels with necrotic endothelium (Figures 11,12,19). Multiple intracytoplasmic (Figure 13) and intranuclear (Figures 20,21) viral inclusion bodies were encountered inside hepatocytes and in mononuclear cells (Figures 8,9,17). Massive fibrosis was substituting the parenchyma showing the picture of chronic hepatitis disease. Liver showed hemorrhages, nucleated red blood cells of camel, and nucleus with condensed chromatin and prominent nucleolus (Figure 14). The kidneys showed degeneration and necrosis of distal and proximal convoluted tubules, hemorrhages were also seen along with blood casts.

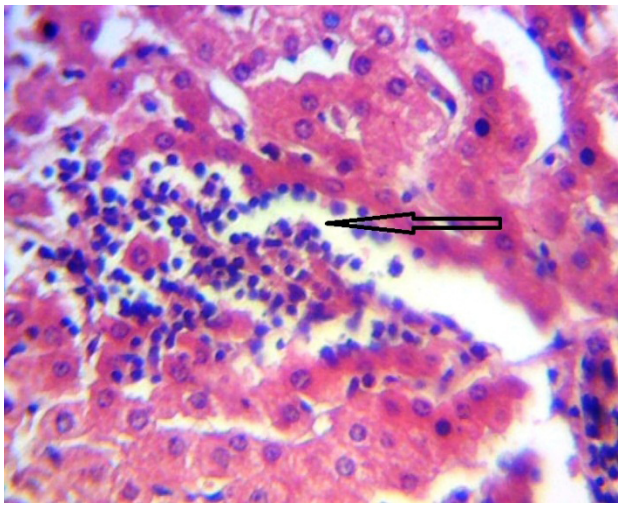


Figure 6

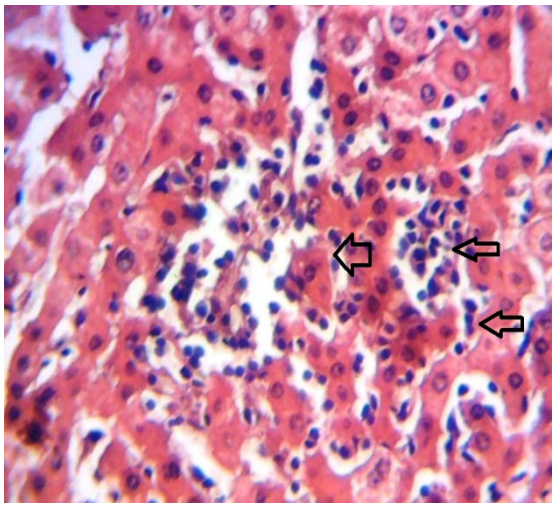


Figure 7

Figures 6,7: Camel, liver: Necrotic foci, activated Kupffer cells. (H&E X 40).

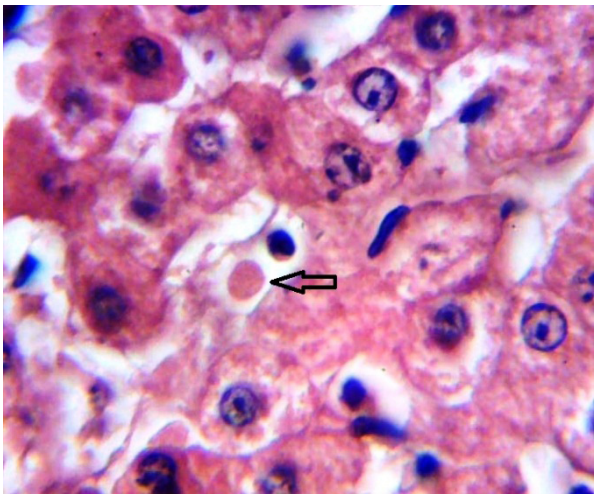


Figure 8

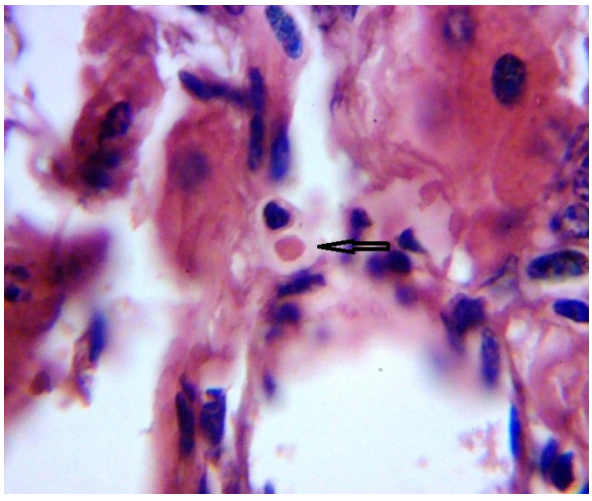


Figure 9

Figures 8,9: Camel, liver: Intracytoplasmic inclusion bodies. (H&E X 100).

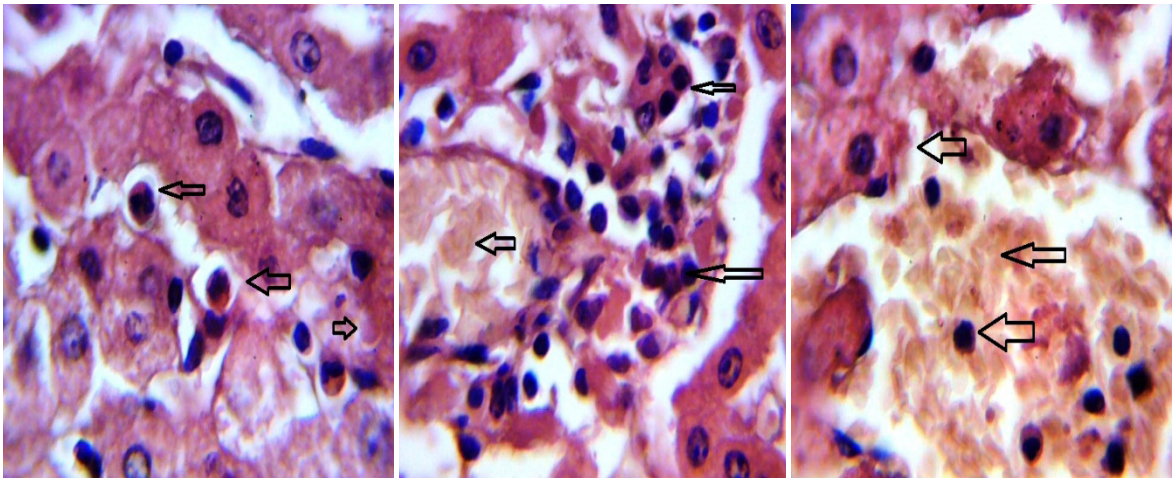


Figure 10 **Figure 11** **Figure 12**

Figures 10-12: Camel, liver: Blood monocytes, lymphocytes infilterations, congested blood vessels. (H&E X 100).

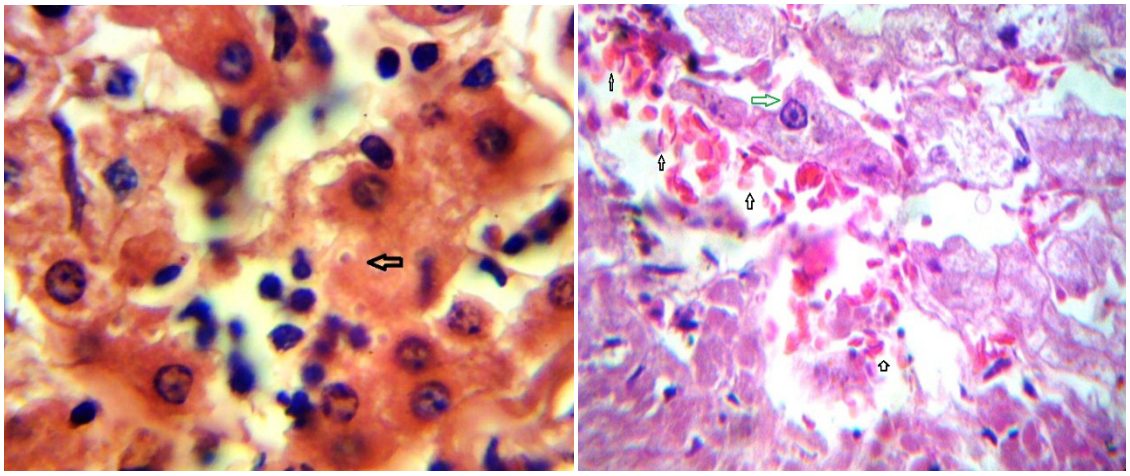


Figure 13 **Figure 14**

Figures 13,14: Camel, liver: Intracytoplasmic and intranuclear inclusion bodies, hemorrhage, lymphocytes infiltration. (H&E X 100).

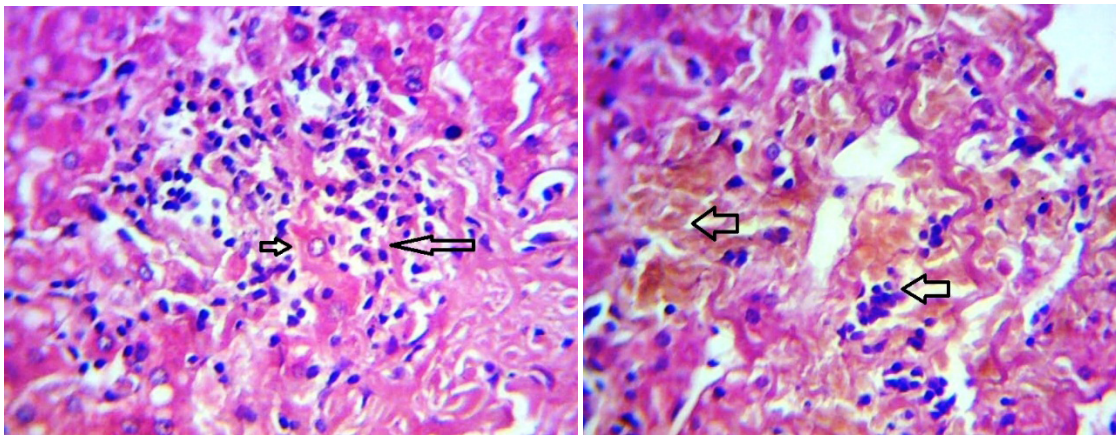


Figure 15 **Figure 16**

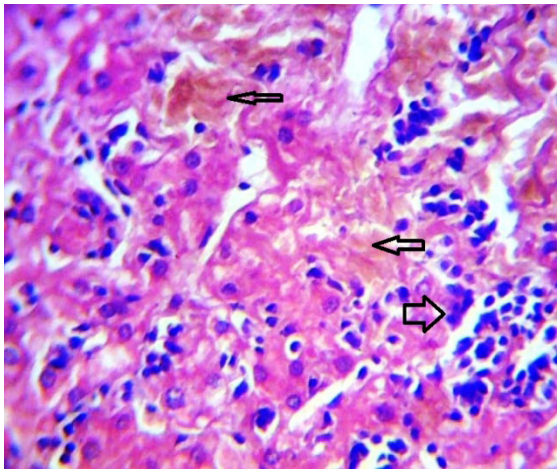


Figure 17

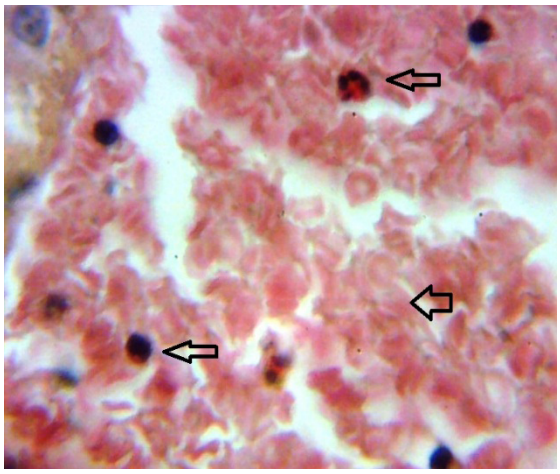
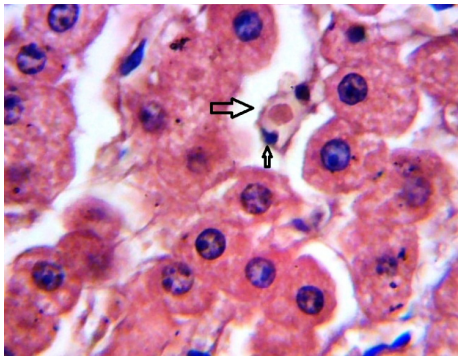


Figure 18

Figures 15-18: Camel, liver: Necrotic foci, proliferated Kupffer cells, hemorrhages (H&E X 40).



Figures 19: Camel, liver: Intracytoplasmic inclusion body inside Kupffer cells (H&E X 100).

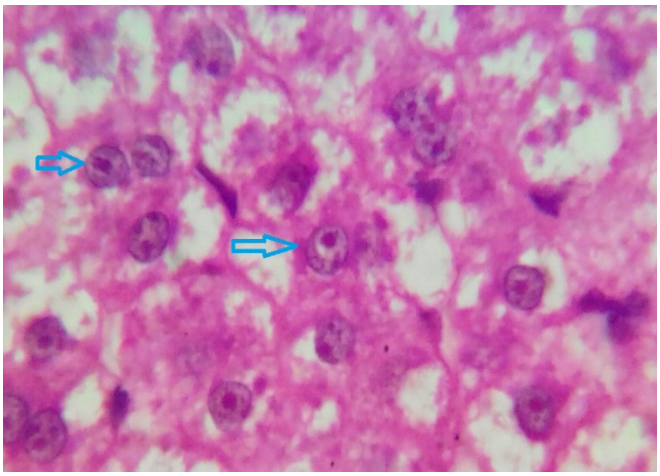


Figure 20: Liver, Camel: Intranuclear viral inclusion bodies in hepatocytes surrounded by hallow zone (Hematoxylin & Fuchsin X 100).

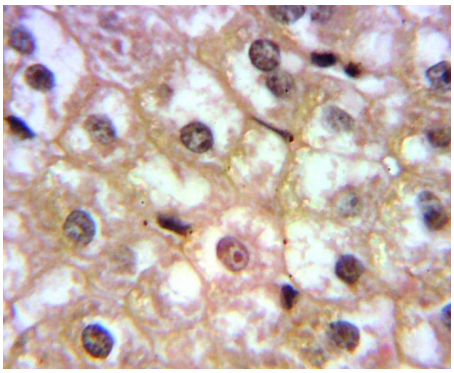


Figure 21: Liver, Camel: Intranuclear viral inclusion bodies in hepatocytes surrounded by hallow zone (Hematoxylin, Phloxine &Tartrazine X 100).

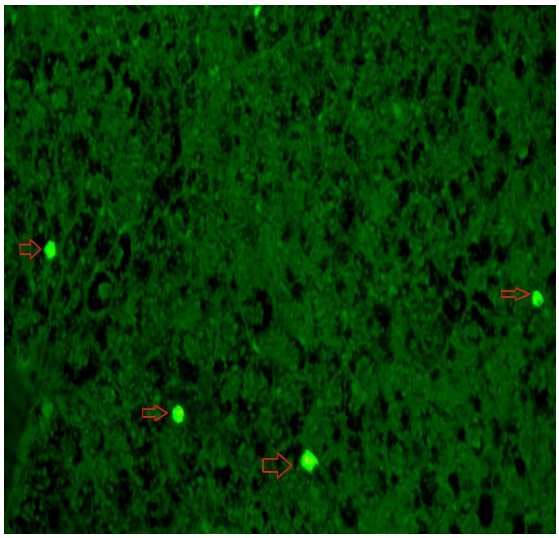


Figure 22

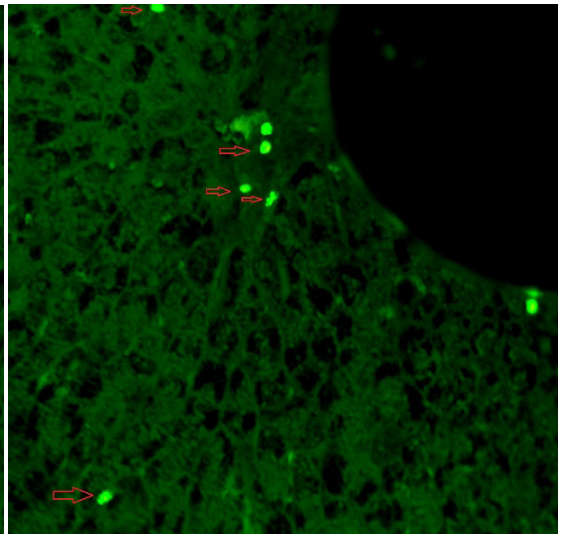


Figure 23

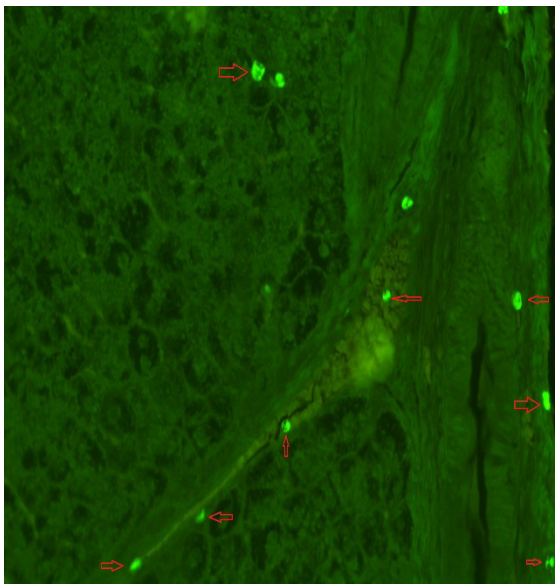


Figure 24

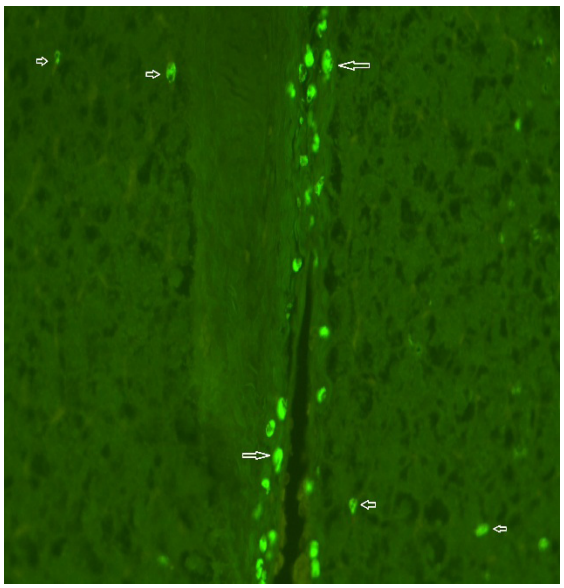
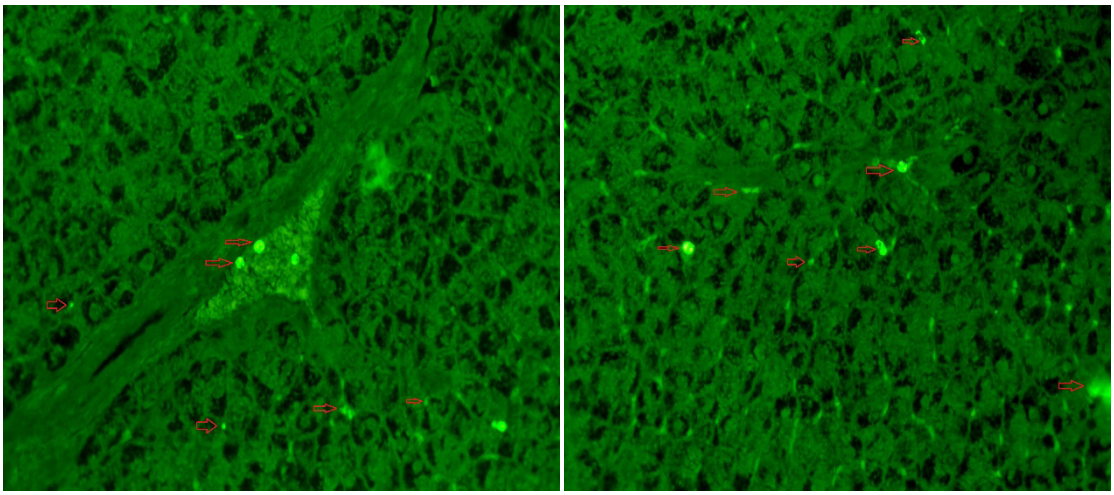


Figure 25



Figures 26 **Figure 27**

Figures 22-27: Liver, Camel: Showing IF positive reactions.

Immunoflurescent [IF]: Positive results were seen in liver of infected camels and buffy coats (Figures 22-27). The best results were encountered with buffy coats and paraffin histological sections. As the viral antigen can visualized at the cellular level by fluorescent microscopy and staining by using antibodies labeled with fluorochromes. The antigen can be obtained from smears of infected tissues, tissue sections or infected monolayer tissue cultures.

Isolation of RVF virus using mice and VERO inoculation: Attempts were carried out for isolation of RVF virus from buffy coats. The results showed failure to isolate the virus even in mice or VERO inoculation.

Results of virus neutralization test: In the present study, the neutralizing antibodies were detected in camel sera (Table 1). Sera which contain antibodies to the virus in question are able to neutralize the aliquot of virus used in the test, thus preventing infection of the cells when they are added to the plate. Where high concentrations of antibody to the virus in question are present in the serum sample, virus neutralization will occur even at high serum dilutions. Conversely, where little or no antibody to the virus is present in the test sample, it will be unable to neutralize the aliquot of infectious virus at the first dilution used in the test. The result of the test is the point at which the serum sample has been diluted such that it is no longer able to neutralize all the virus in the test. This dilution, or its log equivalent, is reported as the titer of the serum tested.

Discussion

Camels belong to the family Camelidae. The Camelidae are separated from the other ruminants into the group Tylopoda (pad-footed). The genus Camelus comprises two species; *Camelus dromedarious*, the dromedary, one-humped or Arabian camel and *Camelus bacterianus*, the bacterian or two-humped camel. The camel can live in hot and arid environments because it possesses an efficient water conservation mechanism which affords it as exceptional tolerance to dehydration. The camel's range in body temperature is such that it may rise by as much as 7 c during the day and hence reduce the need to shed the heat load by sweating and panting. Camels can stay for up to 8-10 weeks without water. camel has the capability of converting poor quality and rangeland forage into milk and meat. Thus, the camel can continue to produce food for people living in a degraded environment and achieves this with minimal impact and disturbances to the ecosystem. It is necessary to learn from pastoralists who have lived with the camel for many years in order to understand fully the diseases of the camel. Although camels are physiologically and anatomically adapted to exploit the scarce desert resources, they are susceptible to a number of diseases. It is generally known that there are no diseases that are specific to the dromedary and all the infections that have so far been reported, are known to exist in other domestic animals. Signs of disease, however, are often missing or obscure. However, the disease was the most important factor in limiting camel production, yet relatively little is known of the pathology of camel disease [16-28].

Our findings prove that RVFV causes chronic disease in infected target. Immune florescence test is the most reliable test, as antigen is seen by microscope (Figures 22-27). It is economic and safe and can used as rapid and highly sensitive technique for RVFV diagnosis. It is more sensitive than RT-PCR and ELISA. It was demonstrated that the indirect sandwich ELISA could be used as a rapid diagnostic tool for the diagnosis of RVF. The test procedures cannot be able to detect the virus of infectivity titre less than 4 $\log_{10}$ TCID $_{50}$/ml [29, 30]. Accordingly, RVFV

biocenosis consists of vertebrates' hosts (man & susceptible animals), arthropods vector, and environmental factors (stagnant water, floods, river basins, lacks, forest, etc. Camels (a target host) are multipurpose domestic animals used for meat, hair and hide production beside transportation. However, Rift Valley Fever Virus (RVFV), is endemic in Egypt because of importation of livestock. However, improper or contaminated vaccines, presence of mosquitoes, vectors, and lack of vector control are considerable reasons as well [31-35]. Camels infected with RVFV can transmit the virus to human in the presence of the mosquitoes' vectors as amplifications cycles need circulation of the pathogens between vertebrates and animals. Our findings are in accordance with what previously discovered in Mauritania during 2010, as an unprecedented outbreak of Rift Valley fever in human and animals was recorded following a heavy rainfall and massive propagation of the mosquitoes' vectors [11].

Camels are infected with RVFV and transmitted it to new lands. The RVFV epidemics in recent years were connected with infected camels. Rift valley fever was isolated from camels during 1978 Imam et al., 1978. Camels are not involved in the vaccination program of RVF vaccines although they played a role in the outbreak of RVF disease in 1977 [36-49]. Unless the general organization of veterinary service of Egypt mention that females and young camels were never imported from foreign countries, this investigation proves that the government gives permission to all kind of camels to enter Egypt. It was observed that, the imported camels from the Sudan as Dabuka most were either pregnant, young suckling calves and their mothers (she-camels) were seen in Shalatin Quarantine area just arrives from the Sudan. This means that they were born during the camel's journey towards Egypt. It also means that those camels are breast feeding and their milk will be sold to humans. Which people believe to cure their illnesses and drink without being treated thermally. All these camels come from outside Egypt through the crossing point in Halaib. And those camels did not enter the quarantine yet. The question to be asked now is: whether all these camels will be injected with the RVF vaccine?, Will all these She Camels be recorded in quarantine as females, ages of young and pregnancy?. The logic answer is (never).

Our findings are in accordance with the well described findings related to the role of Kupffer cells in hepatic affections, however, in RVF hepatic involvement have certainly bad effects in many vital functions in the body. Kupffer Cells (KC) First described by Karl Wilhelm von Kupffer in 1876 as "sternzellen" (star cells or stellate cells). They reside within the lumen of the liver sinusoids. Upon activation KC release various products, these factors regulate the phenotype of KC themselves, and the phenotypes of hepatocytes, endothelial cells and other cells in liver [50]. Kupffer cells play a critical role in the innate immune response; their localization in the hepatic sinusoid allows them to efficiently phagocytize pathogens entering from the portal or arterial circulation. Kupffer cells may play a major anti-inflammatory role. Kupffer cells are also highly poised for clearance of particles, as well as dead and dying erythrocytes and cells in the hepatic parenchyma, from the systemic circulation. Kupffer cells thus comprise the major phagocytic activity of what was classically termed the reticular-endothelial system. While Kupffer cells can be protective in a number of situations, including drug-induced liver injury and toxin-induced fibrosis; adverse regulation in the precise control of inflammatory responses in Kupffer cells can contribute to chronic inflammation in the liver, including alcoholic and nonalcoholic fatty liver diseases (NAFLDs/NASH) [51-59].

Rift Valley Fever virus enters Egypt daily by infected camels. Therefore, Egypt is considered an outlet for the diseases of African countries and is not a source of such epidemics. Entering these large numbers daily has to be predicted with the presence of infection between humans and local animals. International organizations should not encourage the use of live vaccines for the virus. RVFV coexist in elaborate ecological relationships as RVFV circulates between hosts and vectors. Biocenosis a term that describes an ecological group that includes a pathogen and all of its hosts and vectors So that, vector control constitute priority as without a vector, the pathogen could not infect new hosts. Therefore, in this research we recommend not to give the Rift Valley Fever vaccine to the coming camels to Egypt, and if it is vital and very important to use camels for food, it should be sacrificed on the border within the Sudanese territory. Camels should not enter Egyptian territory.

References

1. Daubney R, Hudson JR, Garnham PC (1931) Enzootic hepatitis or Rift Valley fever: an undescribed virus of sheep, cattle and man from East Africa. J Pathol Bacteriol 34: 545-579.
2. Murphy FA, Fauquet CM, Bishop DHL, Ghabrial SA, Jarvis AW, et al. (1995) Family Bunyaviridae. In Virus Taxonomy. Classification and Nomenclature of Viruses. Sixth report of the international committee on taxonomy of viruses. Springer-Verlag, Wien 1995: 300-315.
3. Linthicum KJ, Davies FG, Kairo A, Bailey CL (1985) Rift Valley Fever virus (family Bunyaviridae, genus Phlebovirus). Isolation from Diptera collected during an inter-epizootic period in Kenya. Journal of Hygiene 95: 197-209.
4. Bouloy M (1991) Genome organization and replication strategies. Adv Vir Res 40: 235-266.
5. Wichgers Schreur PJ and Kortekaas J (2016) Single-Molecule FISH Reveals Non-selective Packaging of Rift Valley Fever Virus Genome Segments. PLOS Pathogens 2016.
6. Meegan JM, Hoogstraal H, Moussa MI (1979) An epizootic of Rift Valley fever in Egypt in 1977. Vet. Rec., 105: 124-125.
7. Imam IZE, El-Karamany R, Darwish MA (1979) An epidemic of rift valley fever in egypt. 1. Diagnosis of Rift valley fever in man. Bulletin of the WHO 57: 437-439.
8. Ali RR (1979) Study on Rift Valley fever in camels in Egypt. M.V.Sc. Thesis. Microbiology, Cairo university, Egypt.

9. Hoogstraal H, Meegan JM, Khalil GM, Adham FK (1979) The Rift Valley fever epizootic in Egypt 1977-78; 2. Ecological and entomological studies. Trans R Soc Trop Med Hyg 73: 624-629.

10. Khodeir MH, Khairat E, Gehan KM, Mouaz MA, Saham AS (1993) Immune Status of Rinderpest and Rift Valley fever in Camels. Zag Vet J 21: 317-324.

11. El Mamy ABO, Ould Baba M, Barry Y, Isselmou K, Dia ML, et al. (2011) Unexpected Rift Valley fever outbreak, Northern Mauritania. Emerg Infect Dis 17: 1894-1896.

12. Edwin HL and Nathalie JS (1979) Micro-neutralization test in cell cultures. Diag. Proc. Virol. Rich. & Chlamydial Inf. 5th edition 1979: 108-111.

13. Lillie RD and Fullmer HM (1976) Histopathology Technique and Practical Histochemistry. 4th edition. 1976. McGrew Hill Book Company NY., USA.

14. Payment P and Trudel M (1993) "Methods and Techniques in virology." Text book, Marcel Dekker INC USA.

15. Reed LJ and Muench H (1938) "A simple method of estimating fifty percent endpoint." The American Journal of Hygiene 27: 493-497.

16. Davies FG (1988) Results of the vesicular and scab materials sent by author from Turkana Camel Development Project herd, April 1988. Virology section, VIL Kabete, P.O. Kabete, Nairobi 1988.

17. Gauthier - Pilters H (1984) Aspects of dromedary ecology and ethology. In Cockrill, W.R. (ed.). The camelid vol.1. Scandinavian Institute of African Studies, Uppsula 1984.

18. Grill PJ (1987) introducing the camel. Basic camel keeping of the beginner. Mennonite Central Committee, United Nations Environment Programme 1987.

19. Wilson AJ (1980) Camel diseases in selected areas of Kenya. IPAL Techn. Report No. A-3. Unesco, Nairobi 1980.

20. Walker JB (1962) Notes on the common tick species of East Africa. Second edition. Cooper, McDougall & Robertson (E.A) ltd. Nairobi 1962.

21. Manson IL (1984) Origins, evolution, and distribution of domestic camels. In: Cockrill, W.R. (editor). The Camelid volume 1. Scandinavian Institute of African Studies, Uppsula 1984.

22. Richard D (1979) Dromedary Pathology. In: Cockrill, W.R. (editor). The Camelid volume 1. Scandinavian Institute of African Studies, Uppsula 1979.

23. Richard D (1976) The diseases of the dromedary in Ethiopia. Ethiopian Vet. Bulletin No.2 1976.

24. Wilson RT (1984) The Camel. Longman. London and New York 1984.

25. Wilson RT (1978) Studies on livestock of Southern Darfor, Sudan: Notes on Camels. Tropical Animal Hlth. Prodn 10.

26. Leese AS (1927) A treatise on the one humped camel in health and in disease. Stamford, Haynes and Son 1927.

27. Yagil R (1982) the camel: self-sufficiency in animal protein in drought stricken areas. World Anim. Review 47.

28. Yagil R and Berlyne GM (1987) Glomerular filtration rate and urine concentration in the camel in dehydration. Renal Physiol 1.

29. Abd El Baky MH and Al-Mujalli DM (2004) Serological tests for detection and titration of antibodies to Rift Valley fever in sera of sheep and goats experimentally vaccinated with live attenuated vaccine of smithburn strain (A comparative study). Assiut Vet Med J 50: 128-135.

30. Abd El Baky MH and Al-Mujalli DM (2004) Development of indirect sandwich enzyme linked immunosorbent assay for the detection of Rift Valley fever virus. Assiut Vet Med J 50: 122-127.

31. Terasaki K and Makino S (2015) Interplay between the Virus and Host in Rift Valley Fever Pathogenesis. J Innate Immun 7: 450-458.

32. Moutailler S, Roche B, Thiberge JM, Caro V, Rougeon F, et al. (2011) Host alternation is necessary to maintain the genome stability of Rift Valley Fever virus. PLoS Neglected Tropical Diseases 5: 41.

33. Besselar TG and Blackburn NK (1991) Topological mapping of antigenic sites on the Rift Valley fever virus envelope glycoproteins using monoclonal antibodies. Archives of Virology 121: 111-124.

34. Elliott RM, Schmaljohn CS, Collett MS (1991) Bunyaviridae genome structure and gene expression. Curr Top Micro Immunol, Springer-Verlag, Berlin 1991: 91-142.

35. Giorgi C (1996) Molecular biology of phleboviruses, In RM Eliott, The Bunyaviridae. 1996. Plenum Press, New York 105-128.

36. Arthur RR, El-Sharkawy MS, Cope SE, Botros BA, Oun S, et al (1993) Recurrence of Rift valley fever in Egypt. Lancet 342: 1149-1150.

37. Meegan JM (1979) The Rift Valley fever epizootic in Egypt 1977-78. 1. Description of the epizootic and virological studies. 1979; Trans R Soc Trop Med Hyg 73: 618-623.

38. Meegan JM, Khalil GM, Hoogstrall H, Adham FK (1980) Experimental transmission and field isolation studies implicating Culex pipens as a vector of RVF in Egypt. 1980; Am J Trop Med Hyg 29: 1405-1410.

39. Imam IZE and Darwish MA (1977) A preliminary report on an epidemic of Rift valley fever (RVF) in Egypt. J.E.P.H.Ass 6: 417-418.

40. World health organization (WHO) (1978) Rift valley fever in Egypt. Weekly epidemiological records 53: 107-198.

41. Abdel- Rahim IH, Abdel- Hakim U, Hussein M (1999) An epizootic of Rift valley fever in Egypt in 1997. 1999; Rev. Sci Tech 19: 741-748.

42. World Health Organization (2003) Disease outbreak reported: Rift Valley fever in Egypt. Weekly Epidemiological Record 36: 5.

43. Meegan JM, Niklasson B, Bengtsson E (1979) Spread of Rift valley fever virus from continental Africa. Lancet 1: 1184-1185.

44. Linthicum KJ, Davies FG, Kairo A (1985) Rift Valley Fever virus (family Bunyaviridae, genus Phlebovirus). Isolation from Diptera collected during an inter-epizootic period in Kenya. Journal of Hygiene 95: 197-209.

45. Schmaljohn CS (1996) Fundamental Virology. 3rd edition. 1996. B.N. Fields and lippincott. Raven publishers. Philadelphia, USA 1996.

46. Knipe DM, Howley P. (editors). Fields Virology. PA: Lippincott Williams & Wilkins 2013.

47. Coatzer JAW (1977) The pathology of Rift Valley Fever 1. Lesions occurring in natural cases in new-born lambs. Onderstepoort Journal of Veterinary Research 44: 205-212.

48. Coetzer JA (1982) The pathology of Rift Valley fever. II. Lesions occurring in field cases in adult cattle, calves and aborted fetuses. Onderstepoort J Vet Res 49: 11-17.

49. Findlay GM and Howard EM (1952) Transmission of neurotropic rift valley fever virus to Rats. Annals of Trop. Med. and Parasite 46: 35-37.

50. Bilzer M, Roggel F, Gerbes AL (2006) Role of Kupffer cells in host defense and liver disease. Liver Int 26: 1175-1186.

51. Naito M, Hasegawa G, Takahashi K (1997) Development, differentiation, and maturation of Kupffer cells. Microsc Res Tech 39: 350-364.

52. Rivera CA, Adegboyega P, van Rooijen N, Tagalicud A, Allman M, et al. (2007) Toll-like receptor-4 signaling and Kupffer cells play pivotal roles in the pathogenesis of non-alcoholic steatohepatitis. J Hepatol 47: 571-579.

53. Starkel P, De Saeger C, Strain AJ, Leclercq I, Horsmans Y (2010) NFkappaB, cytokines, TLR 3 and 7 expression in human end-stage HCV and alcoholic liver disease. Eur J Clin Invest 40: 575-584.

54. Stienstra R, Joosten LA, Koenen T, van Tits B, van Diepen JA, et al. (2010) The inflammasome-mediated caspase-1 activation controls adipocyte differentiation and insulin sensitivity. Cell Metab 12: 593-605.

55. Stout RD and Suttles J (2004) Functional plasticity of macrophages: Reversible adaptation to changing microenvironments. J Leukoc Biol 76: 509-513.

56. Tateya S, Rizzo NO, Handa P, Cheng AM, Morgan-Stevenson V, et al. (2011) Endothelial NO/cGMP/VASP signaling attenuates Kupffer cell activation and hepatic insulin resistance induced by high-fat feeding. Diabetes 60: 2792-2801.

57. Taub R (2010) Liver regeneration: From myth to mechanism. Nat Rev Mol Cell Biol 5: 836-847.

58. homson AW and Knolle PA (2010) Antigen-presenting cell function in the tolerogenic liver environment. Nat Rev Immunol 10: 753-766.

59. Zhao XJ, Dong Q, Bindas J, Piganelli JD, Magill A, et al. (2008) TRIF and IRF-3 binding to the TNF promoter results in macrophage TNF dysregulation and steatosis induced by chronic ethanol. J Immunol 181: 3049-3056.