Rift Valley Fever in Chronic Carrier and Renal Manifestations

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Abstract

Background
In Egypt, during September 2017, Rift Valley Fever virus (RVFV) detected in tissue samples of cattle sold from a market, investigations referred to the samples were from imported cattle (country of origin: the Sudan). The natural infection of Rift Valley Fever supposed to cause a certain type of hemorrhagic fever, unless the study of its long-term impact has not been addressed extensively. The predilection site of RVFV was usually referred to the liver and/or the brain, but this research referred to the kidneys. The objectives are to shed light on the pathological changes in the kidneys because this virus affects humans and represents a threat to public health. It also aims to draw attention to the impact of infectious diseases on urinary system.

Material and Methods
50 tissue specimens from imported cattle, trials to isolate RVFV were performed by inoculation of baby mice with tissue homogenates. Agar Gel Precipitation test (AGPT) and the Immuno fluorescence Technique (IF) and reverse transcription RT-PCR were applied. And histopathology sections were prepared, stained and examined microscopically.

Results
The RVFV antigen has seen by fluorescent microscope in the affected kidneys, indicating RVFV distribution inside renal parenchyma. Destructed and necrotic plexus of renal corpuscles were seen surrounded by areas of necrosis. Viral inclusion bodies were detected in cells of renal plexus and tubules. The severity of RVFV infections on renal parenchyma showed changes in kidneys mimics that of the liver. The virus breaking the kidney tissue with the same tactic as the liver.

Conclusions
This study proves that RVFV causes chronic diseases and can be redefined as a virus that causes per-acute, acute, sub-acute, and chronic infections.

Keywords
Rift Valley Fever; Kidneys Diseases

Introduction
This study proves that RVFV causes chronic diseases and carriers are present in the endemic areas. In Egypt, during September 2017, Rift Valley Fever virus (RVFV) was detected in imported cattle from the Sudan. This study was carried out on kidneys of RVFV infected cattle. Rift valley fever (RVF) is a febrile illness, acute or sub acute and zoonotic, that sometimes in severe conditions causes’ hemorrhagic fever and death [1]. Rift valley fever...
Rift Valley Fever virus, an envelope, icosahedral, segmented (Medium, Small, Large), small sized RNA virus belongs to genus Phlebovirus; and the family Bunyaviridae. The RVFV main arthropod hosts are mosquitoes. Man and wide range of mammal’s species are susceptible of variables degree. Cattle are highly susceptible. RVFV that isolated during 1977 epizootic in Egypt showed a spherical viral particle, 100 nm in diameter [2]. The genome of RVFV is composed of three single stranded RNA segments. The large one (L) codes for large polymerase protein, the medium sized segment (M) codes for two major glycoproteins (G1 and G2) while the small segment (S) codes for the N protein. Two non-structural proteins NSm1 and NSs are encoded by the M and S segments respectively [3]. RVFV has three segments, negative or ambisense single stranded RNA (ssRNA) genome. The small (S) segment encodes for a nucleoprotein (N), the non-structural protein (NSs), which supposed to help the virus to evade the host immune system, the polymerase encoded on the large (L) segment. The medium (M) segment encodes two non-structural proteins, NSm1 and NSm2, and two envelope glycoproteins, (Gn and Gc) [4, 5].

A serious epidemic of RVF occurred in Egypt in 1977, in which camels, sheep, cattle, buffaloes and goats were affected [6, 7]. In October 1977, RVF appeared clinically in Egypt, the disease presented itself in the beginning as dengue like fever of man at Belbeis area, Sharkia province [8]. In the 18th October 1977, RVF virus was isolated for the first time from a human case in fever hospital at Zagazig, Sharkia province. The disease was observed clinically in animal in July 1977 in Aswan province which adjoins the Sudan, after that the epizootic had been reported in other provinces [9]. An officially reported outbreak of RVF occurs in Aswan province (Egypt) in man and domestic animals in late May, 1993[10]. Another outbreak occurred in Egypt in Aswan and Assiut provinces between April and August 1997 [11]. And during year 2003 an epizootic of RVFV reported officially in Egypt [12].

The wild vertebrates and invertebrates aid in spreading of RVFV to Egypt during 1977 epizootic [13], where rodents, birds, dogs, and domesticated animals including camels have a significant role in RVF virus epidemiology as reservoirs or amplifying hosts during this epizootic [14].

Rift Valley fever virus (RVFV) lead to cell death by mechanisms of necrosis directed by histiocytes, white blood cells, macrophages, and apoptosis. The disease is fatal in man with unhealthy liver and immuno compromised patients are more liable to the disease. Outbreaks of RVF virus in Africa are characterized by distinct spatial and temporal patterns that are directly related to specific environmental parameters associated with mosquitoes vector that play a role in the maintenance (enzootic) and transmission (epizootic) cycle of the virus [15, 16]. Daubney et al [1] were the first to describe the pathological and postmortem changes of RVF in lambs and sheep in details with more particularly to the liver lesion. The smooth cortical surface of kidneys shows a few small ill-defined haemorrhages. They described the histopathological changes in the kidneys as tubular degeneration [1].

This study aims are to investigate those infectious diseases that cause necrosis is possible factor in the formation of stones in the kidneys and or other renal diseases in human of endemic RVFV countries. Therefore, prove the hidden bad consequences of the previous RVFV infection in the kidneys of victims. As we can consider cattle as model animals for human RVFV infections.

Materials and Methods
1. 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 and VACSERA Egypt.

2. 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 Cattle. Some samples were collected from abattoirs in Cairo, Giza.

3. Samples for Virological Investigation

Sera, whole blood, swaps were collected from live animals; however, cattle in this study were not vaccinated against RVFV. In addition to samples from hepatic blood that collected from the portal veins. Tissue specimens (kidneys) were collected from 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 [17].

4. Histopathology Studies

Tissue specimens were undergoing 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 [18, 19].

5. Tissue Culture Cell Lines

VERO cell line were prepared and provided by VACSEREA Egypt. The VERO cells were used for isolation, adaptation of RVFV, virus titration as well as serum neutralization test (SNT) [19].

6. RVFV Strain

RVFV pantropic Menya Strain (Menya / Sheep/258) was kindly provided by applied research sector VACSEREA, Egypt. RVFV was of infectivity titre in the order of $7.5 \log_{10}$ / ml [19].

7. 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 [20].

8. Trial for RVF Virus Isolation

Mice aged 6 days were inoculated intra-peritoneal by buffy coats and tissue homogenates samples collected from imported cattle 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 were incubated at 37 C and observed daily for 5 days post-inoculation for evidence of cytopathic effect [19].

9. 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 TCID₅₀) 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 Cytopathic 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 [19].

10. 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 cattle were subjected for IF tests. The detection of RVFV antigen in infected and control was applied by direct Immunofluorescence Technique (IF) according to [19]. The control negative, control positive monolayer tissue cultures, and the control tissue sections were kindly provided by VACSEREA Egypt.

11. 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 were kindly provided by VACSEREA 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 complexes to form a precipitate which is trapped in the gel matrix and produces a visible line [19].

12. Reverse Transcription Real Time Polymerase Chain Reaction (RT-PCR)

RVFV genome extraction was performed by Power Prep™ Viral DNA/RNA Extraction Kit Protocol. RT-PCR kits primers and probes were provided by the genetic PCR solutions™, Spain.

Results

This study was conducted on the cattle imported from the Sudan which are usually a free grazing breeds (African breed) living in south Sudan. The environmental
conditions and the endemic status of country of origin are serious factors for the spreading of infectious diseases by living animal transportation. Cattles in the Sudan were not receiving RVFV vaccine. The figures showing cattle drinking dirty water because of lower level management by traders (Figures 1, 2 and 3).

**Figures 1, 2 and 3**: Cows Arrived from the South Sudan to Khartoum Market, Cows are Thirst and Drinking from a Dirty Water

**Figure 1**

**Gross Picture**

Some RVFV infected kidneys were showing congestion on cut sections, the cortical-medullary junction exhibits multiple prominent small whitish stony objects about 3 ml in diameter. Some kidneys seen with hard and serrated large whitish formations, embedded in the cortical-medullary junction. (Figure 4)

**Figure 4**: Cattle, Kidney: Showed Large Whitish Stones Embedded inside the Cortical-Medullary Junction (Arrows)

**Agar Gel Precipitation Tests (AGPT)**

RVFV antigen and the known specific antibodies was completed and form a precipitate which is trapped in the gel matrix and produces a visible line.

**Immunofluorescence Examination**

In kidneys (Figures 5, 6, 7 and 8) the viral inclusion bodies exhibits prominent green florescence (positive reaction). The RVFV antigen was seen inside cytoplasm of histiocytes, macrophages, lymphocytes, endothelial lining of blood vessels, and epithelial lining of renal tubules, and inside the necrotic foci. The RVFV antigen was observed in cells of renal plexus and also inside endothelial lining of Bowman capsules. The viral intracellular inclusion bodies in the histopathological sections were showing strong IF reaction, indicating that inclusion bodies are the aggregates of viral particles (virions) protected inside a membranous formations.

**Histopathology Results**

The microscopic examination (Figures 5 to 54) was showing the RVFV histopathological changes. However the stones formations were seen in some cases beside areas of compressed renal parenchyma causing a large area of necrosis. The special stains for viral inclusion bodies (Phloxine and Tartrazine) were showing numerous intracytoplasmic inclusions in the form of multiple aggregates in the infected cells. Also numerous intranuclear inclusions were observed.
Figures 5, 6, 7 and 8: Fluorescent Microscope: Cattle, Kidneys Showed Positive Reaction of Renal Plexus, Bowman Corpuscle, Histiocytes, Epithelial Cell and Inflammatory Cells Surrounding the Renal Convoluted Tubules having RVFV Antigen (IF X100)

Figure 5

Figure 6

Figure 7

Figure 8

Figure 9: Special Stains: Cattle, Kidney: Showed Intranuclear Viral Inclusion Body in Cell of Renal Plexus (Arrow) (Phloxine and Tartrazine, X100)
Figure 10: Special Stains: Cattle, Kidney: Showed Numerous and Variables in Shapes of Intracytoplasmic Viral Inclusion Body in Cell of Renal Tubules (Arrows) (Phloxine and Tartrazine, X100)

Figure 11: Cattle, Kidneys: Showed Area of Necrogranuloma (Mostly Lymphocytes), Substituted Renal Tissues and Destructed the Renal Parenchyma (Phloxine and Tartrazine, x100)

Figure 12: Cattle, Kidneys: Showed Area of Necrogranuloma (Mostly Lymphocytes), Substituted Renal Tissues and Destructed the Renal Parenchyma (Phloxine and Tartrazine, x40)
Figures 13, 14: Cattle, Kidneys: Showed Congested Blood Vessel, Areas of Necrogranuloma (Mostly Lymphocytes), Substituted Renal Tissues and Destructed the Renal Parenchyma (Arrows) (Phloxine and Tartrazine, x100)

Figure 13

Figure 14

Figure 15: Cattle, Kidneys: Showed Area of Necrogranuloma (Mostly Lymphocytes), Substituted Renal Tissues and Destructed the Renal Parenchyma (Arrows) (Phloxine and Tartrazine, x40)

Figure 15

Figure 16: Cattle, Kidneys: Showed Necrotic Glomerulus, Area of Necrogranuloma (Mostly Lymphocytes), Substituted Renal Tissues and Destructed the Renal Parenchyma (Arrow) (Phloxine and Tartrazine, x100)

Figure 16
Figures 17, 18: Cattle, Kidneys: Showed Hemorrhages, Areas of Necrogranuloma (Necrotic Foci), Substituted Renal Tissues and Destructed the Renal Parenchyma (Arrow) (Phloxine and Tartrazine, x100)

Figures 19, 20: Cattle, Kidneys: Showed Hemorrhages, Areas of Necrogranuloma (Mostly Lymphocytes), Substituted Renal Tissues and Destructed the Renal Parenchyma (Arrow) (Phloxine and Tartrazine, x100)

Figures 21, 22, 23, and 24: Cattle, Kidneys: Showed Hemorrhages, Areas of Necrogranuloma, Substituted Renal Tissues and Destructed the Renal Parenchyma (Arrows) (Phloxine and Tartrazine, x100)
Figures 25, 26, 27 and 28: Cattle, Kidneys: Showed Areas of Necrogranuloma (Mostly Lymphocytes), Substituted Renal Tissues and Destructed the Renal Parenchyma (Arrows) (H and E x40)
**Figures 29, 30:** Cattle, Kidney: Showed Different Shapes of Necrogranulomas (Necrosis) and Inflammatory Cells Substituted the Renal Tubules (Arrows) (H and E x20)

**Figures 31 to 54:** Cattle, Kidneys: Showed Areas of Necrogranuloma (Granulomatous Necrosis), Substituted Renal Tissues and Destructed the Renal Parenchyma, Hemorrhages, Coagulative Necrosis, Proliferation of Histiocytes, Attaching of Renal Parenchyma by Histiocytes and Macrophages, Lymphocytic Infiltrations, Stages of Necrosis inside Areas of Hemorrhage and Different Shapes of Necrotic Foci (H and E stain)
The most outstanding findings are the prominent role of tissue macrophages (histiocytes) in the progress of disease. However, some other phagocytic cells (macrophage) derived from blood monocyte or from peritoneum were also observed attaching the renal tissues and forming the necrotic foci (Figures 47, 48). necrogranulomas (NGs) or the necrotic-granulomas [aggregations of lymphocytes, macrophages, histiocytes, polymorph nuclear cells (PMNC), necrotic parenchyma, cellular debris, red blood cells (RBCs), exudates]. NGs (Figures 11-50) were seen substituted renal cortex and renal tubules. The necrogranuloma (NG) was destructing the renal corpuscles, renal tubules, blood vessels and affect the urethral wall. NGs were seen strangling the renal capsules, invading the plexus, transforming them into necrotic debris. It resembles invading troops, or floods that destruct all things come their ways. Necrotic foci were seen along the whole kidney, it presents around the renal corpuscles, causing necrosis and substitution with necrogranuloma. Coagulative necrosis of renal convoluted tubules and hemorrhages were also seen. Sub capsular necrogranulomas were also observed. Inclusion bodies were seen inside nuclei of histiocytes and councilman like bodies were seen inside some necrotic foci. Inside areas of necrosis there were some renal tubule forming a rosette formation as nuclei were pushed towards the lumen of renal tubules and their cytoplasm seen as a homogenous core in center, cornered by cotton shape formation above basement membranes. Meanwhile, some nuclei were showing intranuclear inclusions.

RT-PCR Findings
Both samples and positive controls samples were giving negative results.

Discussion
We observed through the microscopic examination of kidneys that the Rift Valley virus causes large injuries in its tissues similar to those occurring in the liver as histiocytes of kidneys plays major role in viral pathogenesis. The presence of stones in the kidneys under study and infected with the virus should not be seen as a coincidence, but rather placed under the possibility of causation. We can assume that the salts are deposited on infected tissue and indicate the severity of the changes caused by the Rift Valley virus.

The presence of significant changes in the kidneys infected with the virus in animals is a clear indication of what happens in humans during infection with this virus. Therefore, in countries where this virus is abundant and is permanently present in its environment, whether or not it is the most recent disease, it should be included in the list of infectious diseases affecting the kidneys in humans.

When a person wants to be reassured about his
health, he undergoes routine laboratory tests that include kidney and liver function as well as liver viruses. Routine tests of Rift Valley Fever should also be included in all countries of the world, not just Africa or Arab countries.

We can consider the simple infection of this virus as a causative agent for subsequent complications of vital organs such as kidneys. Therefore, we must look at this matter well so that we protect ourselves from the diseases of the kidneys.

The presence of antibodies in human blood should be considered an infection. The virus, while in the body, caused changes in the kidneys, requiring additional tests for kidneys to treat and reduce the effects of the disease.

In the present study the infected cattle kidneys gross showed several whitish, stiff, irregular, edged, stone like objects (about 2 mm in diameter) embedded in between cortex and medulla. The RVFV antigen are detected by fluorescent microscope in the kidneys, in Bowman capsules, distal and proximal convoluted tubules, histiocytes, macrophages, white blood cells, blood vessels endothelium and inside the necrogranuloma. Focal areas of Coagulative necrosis and hemorrhages were seen in cortex and medulla. Necrotic foci were seen scattered all over the cortex and medulla. Destructed and necrotic plexus of renal corpuscles were seen surrounded by areas of necrosis. Viral inclusion bodies were detected in cells of renal corpuscles and tubules. The pathological examination showed changes in kidneys mimics that of the liver. The clusters of parenchymal cells destructed and eaten by macrophages and white blood cells, as well as histiocytes (tissue resident phagocytes), correspond to what happens in the liver [21-23]. We recommend that a routine test should be devised to examine and diagnose the disease in humans of the African countries.

Natural infection in cattle appears to be severe due to what has been observed in kidneys. Since discovering of RVFV in Kenya, in Rift Valley at Lake Naivasha [1], hepatic necrosis described as the main prominent lesions and liver has been described as a main target of RVFV. Our findings showing that RVFV in kidneys of infected cattle (natural infection) causes severe and destructive necrosis, monitored by infected histiocytes and inflammatory cells resembling the necrogranuloma (necrotic foci) found and described in liver infected with RVFV. Our findings are in accordance with [19, 21, 24-27] who mentioned that RVFV infects macrophages and Dendritic cells (DCs), the cells of innate immune response and the first line of host defense. RVFV is able to replicate in macrophages in vitro and in vivo.

Our findings are in accordance with Daubney et al [1] whom were the first to describe the pathological and postmortem changes of RVF in lambs and sheep in details with more particularly to the liver lesion. In lamb in mild cases the necrotic foci are distributed more or less evenly throughout the whole of the liver beneath the capsule as small, white spots, up to about 1 millimeter in diameter, in the neighborhoods of such small foci a scattered pin-point sub-capsular hemorrhage, reddish black in color however the liver itself is not enlarged. In severe cases most of the liver tissue is affected and the necrotic foci become numerous and coalesce to form a diffuse necrotic lesion and the whole liver may be light yellow. The smooth cortical surface of kidneys shows a few small ill-defined haemorrhages. They described the histopathological changes in the kidneys as tubular degeneration [1]. However, Coatzer [25] describes pyknosis and karyorrhexis of the cellular elements in the glomeruli and a hyalinized appearance of many of these affected glomeruli in a RVFV infected newborn lamb [20].

RT-PCR Findings

The test was using commercial kits which were not providing the sequence and type of primers and probes. So that, RVFV diagnosis by RT-PCR in this research have certain cautions, and negative results may be a false result. Moreover positive results should be in accordance with other laboratory findings. Both samples and positive controls samples were giving negative results (Figure 1, 2 and 3) indicating that this technique is not in accordance with other findings and it is false negatives because reference virus samples (control) were also negative. This point need more investigations, those different primers should prepared from known sequences and by applications of variables trials.
References


