

# An epizootic of Rift Valley fever in Egypt in 1997

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Submitted for publication: 11 November 1998

Accepted for publication: 23 June 1999

## Summary

An epizootic of Rift Valley fever (RVF) occurred in Egypt between April and August 1997. The signs among infected cattle and sheep were high fever, icterus, bloody diarrhoea and abortion. Aborted sheep foetuses and sera from the affected herds were collected in the Aswan and Assiut Provinces, Upper Egypt, for virological and serological examination. A cytopathic effect was detected in Vero cell cultures 48 h after inoculation with the foetal liver and spleen suspensions. The same suspensions caused paralysis and mortalities two to three days post intracerebral injection in mice. The isolated virus was identified using an agar gel precipitation test (AGPT) and a direct fluorescent antibody technique. Serological examination revealed that all tested sheep (57) and cattle (93) gave positive results to serological tests, using a complement fixation (CF), serum neutralisation (SN) and indirect immunofluorescence assay; while only 48 (84.2%) out of 57 sheep sera and 69 (74.2%) out of 93 cattle sera gave positive results using an AGPT. Titration of the serum samples indicated that SN is more sensitive than CF. Importation of infected ruminants, especially camels from the Sudan, is the principal source of infection. Aswan, the nearest Egyptian province to the Sudan, is the focus of RVF virus infection in Egypt. As a result of high insect populations, the epizootics of RVF have usually occurred during the summer in Egypt. Reoccurrence of epizootics from time to time indicates failure of the applied RVF vaccination programme in Egypt.

## Keywords

Egypt – Epizootic – Rift Valley fever virus – Serological techniques – Virus isolation.

## Introduction

Rift Valley fever (RVF) is assumed to have spread from the Rift Valley of Kenya to other regions of Africa where it causes serious disease in man and animals (21). Between 1950 and 1976, at least sixteen major epizootics of RVF have occurred in livestock at various locations in sub-Saharan Africa (11). The RVF virus may be circulating in enzootic areas in parts of central and southern Africa, from where the virus could be carried every few years by movements of infected hosts or infected vectors. The timing of epidemics depends on the coincidence of a number of factors such as large populations of susceptible hosts, the movement of hosts, the spread of vectors and the presence of the virus. Such coincidences may occur more frequently in or near an enzootic area (34). In 1987, the first confirmed outbreak of RVF was reported in West Africa (in Mauritania and Senegal) (39).

Epizootics of RVF in domestic animals are initiated by bites of infected mosquitoes (32). Outbreaks of RVF virus in Africa are characterised by distinct spatial and temporal patterns that are directly related to specific environmental parameters associated with mosquito vectors that play a role in the maintenance (enzootic) and transmission (epizootic) cycles of the virus (19). The virus was isolated from mosquitoes (*Culex zombaensis*, *Mansonia africana* and *Aedes quasitunivittatus*) collected during an outbreak in domestic animals in Kenya (20). *Aedes albopictus* should also be considered a potential vector of RVF virus (37).

Epizootics of RVF in East Africa are associated with an increase in rainfall. However, factors associated with epizootics in West Africa remain unknown (41). Widespread, frequent and persistent rainfall has been a feature during RVF epizootics in Kenya (4). Outbreaks occurred in cattle and

sheep in the rainy season of 1973/1974 and 1977/1978 in Zambia (14). In Mauritania, significant correlation was found between serological evidence of RVF virus infection and the presence of large expanses of stagnant water that provide a suitable habitat for the vectors of the disease (18). In October 1993, at the end of the rainy season, active RVF virus transmission was detected in several locations of southern Mauritania in small ruminants. A high rate of abortion was associated with the virus infection (40).

An important focus of RVF circulation was identified in southern Mauritania. Within this focus, the antibody prevalence rates were 16% (42/262) in goats, 14% (12/84) in sheep, 13% (8/62) in cattle, and 33% (19/58) in camels (30). The few reports of RVF in livestock in Mozambique between 1963 and 1983 indicate the presence of isolated foci (26). An outbreak of RVF was recorded in March 1990 in the district of Fenerive on the east coast of Madagascar, characterised by an abnormally high incidence of abortions and disease in livestock (23); between February and April 1991, an epizootic of RVF was also reported in the central highlands of Madagascar (24).

Routine examination of sera from aborting cattle and sheep for neutralising antibodies to RVF virus provides a regular monitoring system for the virus in Kenya (6). The highest proportion of animals which give seropositive results to RVF virus was detected in the Rift Valley Province in Kenya (17). Serological surveys on cattle in the Congo indicated that 137 out of 515 (27%) serum samples were positive using the enzyme-linked immunosorbent assay (ELISA) for IgG (immunoglobulin G) antibodies to RVF (27). Enzootic maintenance of RVF virus among sheep and goats was detected in two areas of Senegal from 1991 to 1993 (41).

An epizootic of RVF was recorded in Zambia from January to May 1985 (15). Serum samples taken during 1974 and 1978 showed evidence of epizootic Rift Valley fever in Zambia, with more than 80% of animals giving a positive response. A sentinel herd exposed from 1982 to 1986 showed that RVF was present each year – usually at a low level, with 3%-8% of the susceptible cattle seroconverting. Increased activity of the virus was detected in 1985 and 1986, when 20% of the animals were reported to be seropositive (5). Rift Valley fever was found to be distributed widely among cattle in nine districts of Zambia using the indirect immunofluorescence assay (IF) (31).

In the Sudan, an epizootic form of clinical RVF was first recorded in domestic ruminants in 1973, and the epizootic which followed from 1979 to 1981 was remarkable for the high mortalities encountered among local breeds of sheep (especially lambs), goats and cattle (3). The virus circulates across the Sudan, south to north along the Nile Valley with little or no extension to the drier lands to the east and west; ruminants are the primary species involved in virus

maintenance (9). Sera from approximately 2,400 cattle in areas of southern Sudan, collected subsequent to the epizootic of 1979-1981, were positive for antibodies to RVF virus. This suggests an ongoing enzootic status of RVF in the southern Sudan (3).

Prior to the first outbreak in Egypt, RVF had been reported from twenty countries in sub-Saharan Africa (35). In 1977 and 1978, an extensive epizootic of RVF was reported for the first time in Egypt, causing high morbidity and mortality rates in domestic ruminants (1, 16, 22). The Sudan was the most likely origin of the disease and the most probable hypotheses were introduction by infected animals, especially camels, or by insects carried by wind, or a combination of both (34). Another hypothesis suggested the introduction of RVF into Egypt from the Sudan via sheep transported along Lake Nasser (13). A mild wave of the disease was reported in the Aswan Governorate in May 1993, characterised by the ocular form in humans, and abortion in cattle and buffaloes (12). Reintroduction of RVF or other diseases from the Sudan into Egypt will be facilitated by the construction of new roads linking the two countries (13).

Clinically, sheep infected with RVF develop fever, inappetence, vomiting, mucopurulent nasal discharge and bloody diarrhoea. Under field conditions, 90%-100% of pregnant ewes abort, and a mortality rate of 90% is observed in lambs and 20%-60% in adult sheep. In cattle, the disease is somewhat less severe, with mortality rates in calves and cows of 10%-30%; however, pregnant cows always abort (11).

A clinical diagnosis of RVF must be confirmed by serological tests and/or isolation of the virus. The serum neutralisation test (SNT), haemagglutination-inhibition test, and complement fixation test (CFT) are adequate for diagnostic purposes. Fluorescent antibody techniques can detect the presence of the RVF virus in infected tissue cultures within 6 h of inoculation (21).

## Materials and methods

### History of the outbreak

At the beginning of the summer of 1997, a high incidence of abortion was recorded among pregnant sheep and cattle in Upper Egypt. Areas and farms with suspected clinical RVF in Aswan and Assiut Provinces were visited, epidemiological data was collected and clinically affected animals were examined.

### Laboratory diagnosis

#### Materials

A total of 150 serum samples (93 cattle and 57 sheep sera) as well as 6 aborted sheep foetuses were collected from Aswan and Assiut Provinces, Upper Egypt, during a suspected epizootic of RVF in this area. The numbers and locations of

the tested flocks are presented in Table I. The samples obtained were used for virological and serological diagnosis of RVF virus infection. The RVF antigen (a sucrose-acetone extracted from infected mouse liver) was kindly supplied by the United States Naval Medical Research Unit-3 (NAMRU-3), in Cairo. Hyperimmune serum was prepared in rabbits and was also kindly supplied by NAMRU-3.

**Table I**  
**Locations of animals tested and numbers of samples obtained**

Location	Samples collected	
	Serum samples	Aborted fetuses
<b>Aswan Province</b>		
Two dairy cattle farms	30	0
Two sheep flocks	18	1
Individual animals from Daraw community		
– Cattle	9	0
– Sheep	7	1
<b>Assiut Province</b>		
Three dairy cattle farms	42	0
Two sheep flocks	17	2
Individual animals from Mankabad village		
– Cattle	8	0
– Sheep	5	1
Individual animals from Drunka village		
– Cattle	4	0
– Sheep	10	1
<b>Total</b>	<b>150</b>	<b>6</b>

## Methods

### *Virus isolation and identification*

The intracerebral inoculation of the baby mouse was performed using the method described by Easterday (7). The preparation and infection of the Vero (African green monkey kidney) cell cultures was performed according to the technique of El-Nimer (10). For identification of the virus, brain and liver of mice showing paralysis or death after inoculation were tested against RVF virus hyperimmune serum with positive and negative controls by agar gel precipitation test (AGPT) according to the method described by Ouchterlony (28). To perform the direct fluorescent antibody technique (FAT), the inoculated Vero cells were harvested and spotted onto clean dry slides, fixed and stained with a rabbit fluorescein-conjugated immunoglobulin for detection of RVF virus-specific fluorescence.

### *Serological tests*

Four serological tests, namely: AGPT, SNT, CFT and indirect IF were used for the detection of specific antibodies against RVF virus in the serum samples collected. The method described by Nawal was used for the AGPT (25). The SNT employed has been described by Walker *et al.* (38). The CFT was carried out according to Edwin (8) and the indirect IF was

performed following procedures described by Riggs (29); Vero cells, harvested 48 h after infection, were used as antigen.

## Results

### *Clinical and epidemiological studies*

A high incidence of abortion was observed among pregnant sheep and cattle with high mortalities in young lambs and calves during the summer of 1997 in Upper Egypt. Affected areas and farms in the Aswan and Assiut Provinces were visited for collection of epidemiological data and clinical examination of animals. Sheep and cattle showed inappetence, mucopurulent nasal and ocular discharges, high fever and abortion. Some affected cases showed jaundice and bloody diarrhoea.

Clinical examination revealed that the disease was more severe in sheep than in cattle and in young animals than in adults. The abortion rate in pregnant ewes was approximately 60%-70% and in pregnant cows approximately 30%-40%. A mortality rate of approximately 50%-60% was recorded in young lambs, 25%-35% in adult sheep, 25%-30% in calves and 10%-20% in adult cattle.

At the time when the outbreak occurred (during the summer months, from April to August), a large population of insect vectors was present in Egypt. The epizootic originated in the Aswan Province, mainly due to continuous importation of infected ruminants from the Sudan into Aswan.

## Laboratory diagnosis

### *Virus isolation and identification*

Cytopathic effect (CPE) was detected in Vero cell cultures within 48 h of inoculation with the foetal liver and spleen suspensions. The same suspensions caused paralysis and mortalities 2-3 days post intracerebral injection in mice. Precipitation bands were observed in the AGPT using 10% suspensions of brain and liver of mice which showed paralysis or death after inoculation. Using the FAT, virus-specific fluorescence was seen in the Vero cells after staining with rabbit fluorescein-conjugated immunoglobulin. Table II presents the results of isolation and identification of RVF virus.

**Table II**  
**Isolation and identification of Rift Valley fever virus**

Tissue cultures and experimental animals	Route of inoculation	Death or cytopathic effect	Virus identification	
			AGPT	FAT
Suckling mice	intracerebral	2-3 days	positive	not tested
Vero cells		2 days	not tested	positive

AGPT : agar gel precipitation test

FAT : fluorescent antibody technique

### Serological examination

The results of serological testing of cattle and sheep sera for detection of RVF antibodies using AGPT, indirect IF, CFT and SNT are presented in Table III.

## Discussion

Rift Valley fever virus infection was identified for the first time in the Rift Valley of Kenya in 1930 and 1931 (2). Epizootics and/or serological evidence of RVF virus infection had been reported previously in many countries of sub-Saharan Africa, such as Kenya (4, 6, 17), Mauritania (18, 30, 39, 40), Senegal (39, 41), the Sudan (3, 9), the Congo (27), Zambia (5, 14, 15, 31), Mozambique (26) and Madagascar (23, 24).

In Egypt, an extensive epidemic of RVF was reported for the first time in 1977 and 1978 (1, 16, 22). The Sudan was the most likely origin of the disease, which was introduced by infected animals and/or insects carried by the wind (13, 34). A mild wave of the disease was also reported in the Aswan Governorate in May 1993 (12). This study reports an epizootic of RVF in the Aswan and Assiut Provinces of Egypt between April and August 1997. The epizootic commenced in Aswan, which is the nearest province to the Sudan. The authors therefore suggest that the Sudan was also the origin of this outbreak. Continuous importation of viraemic ruminants, especially camels, from the Sudan is the main source of infection. Gad *et al.* suggested that the reintroduction of RVF from the Sudan into Egypt will be facilitated by the construction of new roads linking the two countries (13).

Epizootics of RVF are usually associated with an increase in rainfall in the countries of Africa where the disease is endemic (4, 14, 40, 41). Epidemics of RVF in domestic animals are initiated by bites of infected mosquitoes (12, 32). The virus was isolated from mosquitoes collected during an outbreak in domestic animals in Kenya (20). Epizootics of RVF usually occur in the summer in Egypt due to the presence of large populations of insects during the hot season. The present epizootic occurred in the summer season of 1997.

Clinically, the authors found that the outbreak was characterised by a high incidence of abortion among pregnant ewes and cows as well as high mortalities among young lambs and calves. These clinical symptoms were in agreement with those mentioned by Fenner *et al.* (11).

A clinical diagnosis of RVF must be confirmed by serological tests and/or isolation of the virus (21). Infection was diagnosed by isolation and identification of the causative virus in addition to detection of the specific antibodies in sera of affected cattle and sheep. The virus was isolated by inoculation of specimens in Vero cell cultures where CPE was detected 48 h post-inoculation. The isolated virus was identified using AGPT and direct FAT. Fluorescent antibody techniques can detect the presence of the virus in infected tissue cultures within 6 h of inoculation (21).

Serological diagnosis of the disease revealed that 100% of the 57 sheep and 93 cattle sera tested were serologically positive using SNT, indirect IF and CFT, while only 48 (84.2%) of 57 sheep sera and 69 (74.2%) of 93 cattle sera were positive using AGPT. This result indicates that SNT, indirect IF and CFT have equal sensitivity for the serological diagnosis of RVF, while AGPT is less sensitive for detection of antibodies to the RVF virus (Table III). The SNT, CFT and haemagglutination inhibition test are adequate for the diagnosis of RVF (21). A comparative study of five serological tests for the detection of RVF viral antibodies in sheep sera, demonstrated that the plaque reduction neutralisation test (PRNT), haemagglutination inhibition and ELISA were of the same sensitivity, whilst IF was less sensitive than PRNT, and CFT was the least sensitive (33).

In the study conducted by the authors, titration of the serum samples indicated that SNT is also more sensitive than CFT. In a comparative study of nine serological techniques used to monitor the response to infection with RVF virus in three sheep, the earliest antibody response was detected in one sheep on day three using a PRNT, which also produced the highest titres (36).

**Table III**

**The results of serological tests used for detection of Rift Valley fever antibodies in sheep and cattle sera**

Species	No. of sera tested	AGPT		Indirect IF		CFT titres				SNT titres			
		Positive	Negative	Positive	Negative	8	16	32	64	40	80	160	320
Sheep	57	48	9	57	0	9	9	15	24	6	3	15	33
Cattle	93	69	24	93	0	3	9	30	51	12	12	39	30

AGPT : agar gel precipitation test

IF : immunofluorescence assay

CFT : complement fixation test

SNT : serum neutralisation test

## Conclusion

The present study concluded that the haphazard importation of animals infected with RVF from the Sudan and failure of the locally applied RVF vaccination programme are the main causes of the reoccurrence of RVF epizootics in Egypt. Continued outbreaks of RVF among domestic ruminants, in 1977, 1978 and 1993, in addition to 1997, indicate that the virus has become enzootic in Egypt. To control the disease in Egypt, the authors suggest the following measures:

- prevention of introduction of ruminants infected with RVF, especially camels, from northern Sudan into southern Egypt (Aswan Province)

- avoiding importation of ruminants from countries where RVF is enzootic
- annual vaccination of all ruminants (camels, cattle, buffaloes, sheep and goats) with an effective RVF vaccine
- trials for control of insect vectors especially in the summer season
- the intensive broadcast of educational television programmes to inform farmers and owners of animals of the importance of the vaccination programmes.

## Épizootie de fièvre de la Vallée du Rift en Égypte en 1997

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### Résumé

Une épizootie de fièvre de la Vallée du Rift est survenue en Égypte entre avril et août 1997. Les bovins et ovins infectés présentaient les signes suivants : forte fièvre, ictère, diarrhée sanguinolente et avortement. Des prélèvements d'avortons et de sérums d'ovins appartenant à des élevages infectés des régions d'Assouan et d'Assiout, Haute Égypte, ont été soumis à des examens virologiques et sérologiques. Un effet cytopathogène a été décelé dans les cultures cellulaires Vero, 48 heures après inoculation de suspensions de foie et de rate de fœtus. Ces mêmes suspensions injectées à des souris par voie intracérébrale ont provoqué la paralysie et la mort. L'identification du virus isolé a été réalisée par le test d'immunodiffusion en gélose ainsi que par immunofluorescence directe. Tous les sérums ovins (57) et bovins (93) examinés ont donné des résultats positifs aux trois épreuves sérologiques (fixation du complément, séroneutralisation et immunofluorescence indirecte) ; en revanche, seuls 48 (84,2 %) des 57 sérums ovins et 69 (74,2 %) des 93 sérums bovins contenaient des anticorps précipitants. À en juger par les titrages sérologiques, la séroneutralisation est plus sensible que la fixation du complément. L'importation de ruminants infectés, notamment de dromadaires en provenance du Soudan, est la principale cause d'introduction du virus. Assouan, la province égyptienne la plus proche du Soudan, est le foyer du virus de la fièvre de la Vallée du Rift en Égypte. Du fait de la présence d'importantes populations d'insectes en été, les épizooties de fièvre de la Vallée du Rift en Égypte se produisent d'ordinaire pendant cette saison. La réapparition périodique de la fièvre de la Vallée du Rift révèle les insuffisances du programme de vaccination contre cette maladie en Égypte.

### Mots-clés

Égypte – Épizootie – Isolement du virus – Techniques sérologiques – Virus de la fièvre de la Vallée du Rift.

## Episodio epizoótico de fiebre del Valle del Rift en Egipto en 1997

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### Resumen

Entre abril y agosto de 1997 se declaró en Egipto una epizootia de fiebre del Valle del Rift. Los bovinos y ovinos infectados presentaban cuadros con fiebre alta, ictericia, diarrea hemorrágica y abortos. A fin de realizar un estudio virológico y serológico, se recogieron sueros y fetos ovinos abortivos de los rebaños afectados en las provincias de Asuán y Asiuat (Alto Egipto). Cuarenta y ocho horas después de inocular cultivos de células Vero con suspensiones de hígado y bazo fetales, se observó la aparición de un efecto citopático. La inyección intracerebral de las mismas suspensiones a ratones provocaba la parálisis y muerte de los animales entre dos y tres días después. Para identificar el virus aislado se utilizaron una prueba de precipitación en gel de agar y una técnica de inmunofluorescencia directa. El análisis serológico, realizado con técnicas de fijación del complemento, neutralización sérica e inmunofluorescencia indirecta, arrojó resultados positivos para todos los ovinos y bovinos estudiados (57 y 93 respectivamente); la aplicación de la prueba de precipitación en gel de agar, en cambio, ofreció un resultado positivo en 48 (84,2%) de los 57 sueros ovinos y 69 (74,2%) de los 93 sueros bovinos. La titulación de las muestras de suero puso de relieve que la técnica de neutralización posee mayor sensibilidad que la de fijación del complemento. La importación de rumiantes infectados, en especial de camellos procedentes de Sudán, constituye la principal fuente de infección. Asuán, la provincia más cercana a Sudán, es el foco a partir del cual se extiende la infección por Egipto. A consecuencia de las elevadas poblaciones de insectos propias del verano, las epizootias de fiebre del Valle del Rift en Egipto suelen producirse en esa época del año. La aparición reiterada de epizootias pone de manifiesto el fracaso del programa de vacunaciones contra la fiebre del Valle del Rift que ha venido aplicándose en Egipto.

### Palabras clave

Aislamiento de virus – Egipto – Epizootia – Técnicas serológicas – Virus de la fiebre del Valle del Rift.

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