Epidem. Inf. (1987), **99**, 517–522
Printed in Great Britain

Rift Valley fever: a sero-epidemiological survey among pregnant women in Mozambique

By B. NIKLASSON

Department of Virology, National Bacteriological Laboratory, S-105 21 Stockholm, Sweden

J. LILJESTRAND

Department of Obstetrics and Gynecology, Central Hospital, S-371 85 Karlskrona, Sweden

S. BERGSTRÖM

Department of Obstetrics and Gynecology, Central Hospital, Maputo, Mozambique

AND C. J. PETERS

US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21701, USA

(*Accepted 19 May 1987*)

SUMMARY

Rift Valley fever (RVF) causes abortion in sheep and cattle. However, the teratogenic and abortogenic potential of RVF in humans is not known. Sera from a total of 1163 pregnant women in Mozambique were tested for RVF virus antibodies by ELISA and 28 (2%) were found to be positive. Mothers experiencing fetal death or miscarriage (155) had the same RVF virus antibody prevalence as those with normal deliveries. Analysis of maternity histories showed some indication of increased fetal wastage among women positive for RVF virus antibody.

The ELISA used in this study was compared with a plaque reduction neutralization test and found to be equally sensitive and specific for the detection of RVF virus IgG antibodies.

INTRODUCTION

Rift Valley fever (RVF) is an arthropod-borne viral disease affecting sheep, cattle and man in many areas of Africa (Peters & Meegan, 1983). Field studies and laboratory investigations indicate that RVF causes abortion in almost all pregnant ewes and cows infected (Easterday, Murphy & Bennett, 1962*a*, *b*). RVF in humans used to be considered to be a non-fatal illness (Daubney, Hudson & Garnham, 1931; Francis & Magill, 1935; Smithburn *et al.* 1949; Murphy & Easterday, 1961). However, in 1975 the occurrence of fatal haemorrhagic fever and encephalitis after RVF was documented in South Africa and Zimbabwe (van Velden *et al.* 1977; Swanepoel, Manning & Watt, 1979). During RVF epidemics in

Egypt in 1977 and 1978, a large number of human fatalities were reported (Laughlin *et al.* 1979). The teratogenic or abortogenic potential of RVF for humans has not been examined although one study in Egypt during the 1978 epidemic failed to show any correlation between RVF infection and abortion (Abdel-Aziz, Meegan & Laughlin, 1980). No major RVF epidemics or epizootics have been reported from Mozambique. To date, only a limited outbreak among cattle in 1969 has been reported (McIntosh, 1972).

Liljestrand (1985) made a nation-wide study of maternal morbidity in Mozambique during 1981–83. We took the opportunity to test sera collected during this survey to investigate whether RVF virus infects man in Mozambique and whether it is of any importance as a cause of stillbirth and intrauterine death in pregnant women. A recently developed enzyme-linked immunosorbent assay was used as a screening method for antibodies to RVF virus and compared with results obtained using a plaque reduction neutralization test (Niklasson *et al.* 1984).

MATERIALS AND METHODS

Serological specimens

During a nation-wide maternal morbidity study in 1981–83 sera were collected from 801 pregnant women at 8 sites in 8 of the 10 provinces of Mozambique (Liljestrand, 1985).

Information on area of residence, age, number of previous stillbirths and livebirths and socioeconomic data (e.g., if the family had domestic animals) was collected.

Additionally, during a 10-week period (March–May 1983) all maternity units in Maputo, the capital city, were asked to refer all cases of intrauterine death or stillbirth to Maputo Central hospital. A total of 159 cases were studied and sera were available from 155 of the parturients. At the same time sera were collected from a control group of 207 women delivering liveborn babies in the suburban maternity units of Maputo.

Serological tests

An enzyme-linked immunosorbent assay (ELISA) was used for detection of specific RVF virus (RVFV) IgG. Full details on the preparation of reagents and performance of the test are documented by Niklasson *et al.* (1984). In short, a β-propiolactone-inactivated, sucrose-acetone-extracted, suckling mouse liver RVFV antigen was captured by mouse RVFV antibodies adsorbed to polystyrene plates. The test sample (human serum) was then added (diluted 1/100), and the binding of specific antibodies detected by alkaline phosphatase-conjugated swine anti-human IgG.

The Entebbe strain of RVF virus (Randall *et al.* 1962) was used as antigen in the ELISA.

In the plaque reduction neutralization test (PRNT) sera were serially diluted in fourfold steps from 1/10 and added to previously titrated Zagazig (ZH) 501 strain of RVFV (Meegan, 1979). The mixtures (containing 40–50 PFU) were incubated for 60 min at 37 °C and then seeded into tissue culture plates containing 2–4 day-old confluent Vero cell monolayers. After adsorption, the cells were overlaid with

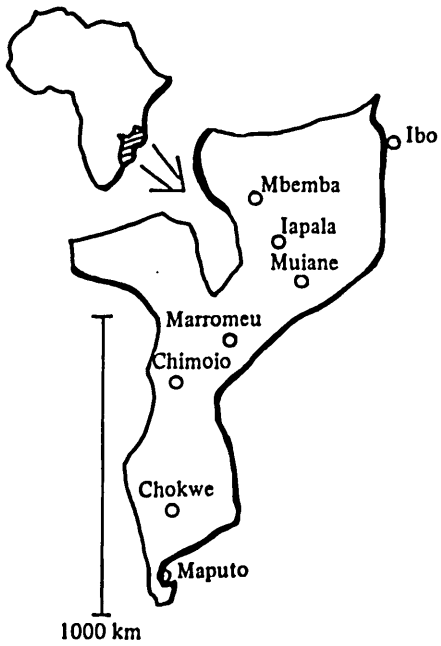

Fig. 1. Map of Mozambique showing the geographical locations of the study sites.

agar and incubated for 3 days at 37 °C in 5% CO_2 followed by a second overlay containing neutral red. Plaques were counted the following day. The dilution of serum giving an 80% reduction in plaques was taken as the virus neutralization titres. Full details of the PRNT test are given in the paper by Niklasson *et al.* (1984).

All sera were sent frozen to Sweden and USA and stored at −20 °C until tested.

RESULTS

A total of 1163 women were tested by the IgG ELISA and 28 (2%) were found to be positive. The area of residence and the results of the assays are given in Table 1 and on the map, Figure 1. RVFV antibodies were found at all the sites investigated except two – Ibo, an island close to the coast, and Muiane, a mining village. In the remaining sites antibody prevalence rates ranged from <1% (2/241) in Iapala to 7% (5/73) in Marromeu. The areas with the highest antibody prevalence (Maputo, Chokwe and Marromeu) were all on the coast or close to a riverbank. High antibody prevalence rates also coincided with presence of cattle or wild buffalos in the area. Information on household animals was available for 698 of the women tested and were present in 52%. Information was also available for 12 of the RVF positive women and 6 (50%) had household animals.

Among the 155 women who had stillbirths, RVF antibodies were detected in 4 sera (3%) while 6 RVF positive sera were found in the control group of 207 women (3%).

The maternity history was available for 990 (85%) of the total population investigated (including 21 of 28 RVF-positive women). The average number of

https://doi.org/10.1017/S0950268800068011 Published online by Cambridge University Press

Table 1. *RVF virus antibody prevalence at different geographical locations in Mozambique*

Name of area	Positive/number of specimens (%)	Type of area
Chokwe	5/90 (6)	Agricultural village at a river bank; large herds of cattle
Chimoio	3/219 (1)	Provincial capital (urban); cattle
Marromeu	5/73 (7)	Agricultural village at a river bank; large herds of wild buffalo
Iapala	2/241 (1)	Agricultural rural area; no cattle
Mbemba	1/32 (1)	Rural; no cattle
Ibo	0/34 (0)	Island; no cattle
Muiane	0/50 (0)	Mining-village; no cattle
Maputo	2/62 (3)	Capital of Mozambique; coastal city; cattle
Maputo (stillbirth)	4/155 (3)	
Maputo (control)	6/207 (3)	
Total	28/1163 (2)	

Table 2. *Childbirth history of women with or without serological evidence of RVF*

RVF antibody status	Positive	Negative	Total
Women	21	969	990
Pregnancies	65	3124	3189
Stillbirth (% of pregnancies)	10 (15)	209 (6)	219 (7)
Women with 1 or more stillbirth (%)	5 (24)	143 (15)	148 (15)

pregnancies was similar regardless of antibody status, but the proportion which ended in fetal death was higher in RVF-positive mothers (15% vs 7%, $P < 0{\cdot}01$, Table 2). A single antibody-positive mother was responsible for 5 of the 10 stillbirths. The proportion of gravid women suffering one or more stillbirths is higher in the antibody positive group (24%) than in the negative women (15%) but this was not significantly different.

All sera found positive by ELISA were tested by PRNT and confirmed as positive. One serum had a titre of 10, one serum a titre of 20 and all the remaining had titres of 160 or greater.

A random sample of 200 sera negative by ELISA was also tested and all were found to be negative by PRNT.

DISCUSSION

There is evidence of RVF activity in most sub-Saharan areas of Africa (Peters & Meegan, 1983). However the only published data from Mozambique concerns a limited outbreak among cattle in 1969 (McIntosh, 1972). The concordant ELISA and PRNT antibody titres presented here establish that humans had also been infected. Since all seropositive individuals were born before 1969, we cannot distinguish between endemic transmission and the consequences of the recognized

epizootic. Residents of areas near water sources where cattle were kept had higher levels of antibody prevalence, although current possession of household domestic animals did not seem to be important. Studies in Kenya suggest that RVFV is maintained in nature through transovarial transmission in floodwater *Aedes* mosquitoes and that further virus amplification occurs in particularly rainy years when arthropods feed on cattle (Linthicum *et al.* 1985). These findings may explain our results.

Mothers experiencing fetal death or miscarriage had the same RVFV antibody prevalence as those with normal deliveries. Samples were obtained at the end of the rainy season when RVF would be most likely to occur, although this was not an unusually wet year and there was no evidence of RVFV activity. When childbirths were analysed, there was some evidence for increased fetal wastage among RVFV antibody-positive women although this was only suggestive.

The ELISA used in this study proved to be a sensitive and specific method of screening for RVF antibodies. Earlier studies performed using sera after RVF-vaccination have shown the ELISA to be more sensitive than complement fixation and haemagglutination inhibition tests and almost as sensitive as the PRNT (Niklasson *et al.* 1984). It is well established that other viruses in the phlebotomus fever group cross-react with RVF in CF and HI while a lower level of cross-reactivity has been seen by PRNT (Shope *et al.* 1981). In this serum collection, PRNT titres of 10 or more were also ELISA positive and all ELISA positives had PRNT titres of 10 or greater. However, the sensitivity and specificity can only be determined for this epidemiological situation. In other geographic areas the presence of serologically-related viruses may cause problems using the ELISA.

The present study has documented RVF antibodies in humans from different parts of Mozambique indicating that the disease is a potential health hazard in the country. There is no evidence presented proving that RVFV infection is an aetiologic agent of stillbirth but further studies, preferably during RVF epidemics, are necessary to evaluate the role of RVF in human abortion.

The views of the authors do not purport to reflect the position of the Department of the Army or the Department of Defense.

REFERENCES

Abdel-Aziz, A. A., Meegan, J. M. & Laughlin, L. W. (1980). Rift Valley fever as a possible cause of human abortions. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **74**, 685.

Daubney, R., Hudson, J. R. & Garnham, P. C. (1931). Enzootic hepatitis or Rift Valley fever. An undescribed virus disease of sheep, cattle and man from east Africa. *Journal of Pathology and Bacteriology* **34**, 545–579.

Easterday, B. C., Murphy, L. C. & Bennett, D. G. (1962*a*). Experimental Rift Valley fever in calves, goats and pigs. *American Journal of Veterinary Research* **23**, 1224–1230.

Easterday, B. C., Murphy, L. C. & Bennett, D. G. (1962*b*). Experimental Rift Valley fever in lambs and sheep. *American Journal of Veterinary Research* **23**, 1231–1240.

Francis, T. & Magill, T. P. (1935). Rift Valley fever: a report of 3 cases of laboratory infection and the experimental transmission of the disease to ferrets. *Journal of Experimental Medicine* **62**, 433–448.

LAUGHLIN, L. W., MEEGAN, J. M., STRAUSBAUGH, L. J., MORENS, D. M. & WATTEN, R. H. (1979). Epidemic Rift Valley fever in Egypt; Observation of the spectrum of human illness. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **73**, 630–633.

LILJESTRAND, J. (1985). Maternal morbidity in Mozambique. M.D. Thesis. University of Uppsala, Sweden.

LINTHICUM, K. J., DAVIES, F. G., KAIRO, A. & BAILEY, C. L. (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.

MCINTOSH, B. M. (1972). Rift Valley fever: vector studies in the field. *Journal of the South African Veterinary Medical Association* **43**, 391–395.

MEEGAN, J. M. (1979). The Rift Valley fever epizootic in Egypt 1977–1978. 1. Description of the epizootic and virological studies. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **73**, 618–623.

MURPHY, L. C. & EASTERDAY, B. C. (1961). Rift Valley fever: A zoonosis. *Annual Proceedings United States Livestock Sanitary Association* **65**, 397–412.

NIKLASSON, B., GRANDIEN, M., PETERS, C. J. & WOOD, O. (1984). Detection of human immunoglobulins G and M antibodies to Rift Valley fever virus by enzyme-linked immunosorbent assay. *Journal of Clinical Microbiology* **19**, 225–229.

PETERS, C. J. & MEEGAN, J. M. (1983). Rift Valley fever. In *CRC handbook series in zoonoses, section B: viral zoonoses* (ed. G. W. Beran). Vol. 1, pp. 403–420. Boca Raton, Fla., USA: CRC Press Inc.

RANDALL, R., GIBBS, C. J., JR., AULISIO, C. G., BINN, L. N. & HARRISON, V. R. (1962). The development of a formalin-killed Rift Valley fever virus vaccine for use in man. *Journal of Immunology* **89**, 660–671.

SHOPE, R. E., MEEGAN, J. M., PETERS, C. J., TESH, R. B. & TRAVASSOS DA ROSA, A. A. (1981). Immunologic status of Rift Valley fever virus. Contribution to *Epidemiology and Biostatistics* (ed. T. A. Swartz, M. A. Klingberg and N. Goldblum) **3**, pp. 42–52. Basel: S. Karger.

SMITHBURN, K. C., MAHAFFY, A. F., HADDOW, A. J., KITCHEN, S. F. & SMITH, J. F. (1949). Rift Valley fever: accidental infections among laboratory workers. *Journal of Immunology* **62**, 213–227.

SWANEPOEL, R., MANNING, B. & WATT, J. A. (1979). Fatal Rift Valley fever of man in Rhodesia. *Central African Journal of Medicine* **25**, 1–8.

VAN VELDEN, D. J. J., MEYER, J. D., OLIVER, J., GEAR, J. H. S. & MCINTOSH, B. (1977). Rift Valley fever affecting humans in South Africa: a clinico-pathological study. *South African Journal of Medicine* **51**, 867–871.