J. Hyg., Camb. (1984), **93**, 629–637
Printed in Great Britain

Preliminary survey of domestic animals of the Sudan for precipitating antibodies to Rift Valley fever virus

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(*Received* 12 *March* 1984; *accepted* 17 *June* 1984)

SUMMARY

In a preliminary seroepidemiological survey a total of 780 serum samples derived from various domestic animals of the Sudan were examined for Rift Valley fever (RVF) virus precipitating antibodies. The incidence was approximately 34·3% in sheep, 33·2% in cattle, 22% in goats, 7·9% in camels and 4% in donkeys. The findings indicated that RVF is mainly prevalent in the rich savanna areas of the south as well as the irrigated areas close to the Nile in the north.

Circumstantial evidence suggests that the detected antibodies were induced by a long-standing cryptically cycling infection and that resurgence of extensive epizootics is unlikely although limited outbreaks may occur. It is concluded that RVF virus circulates across the country in a south–north range along the Nile Valley with little or no extension to the drier lands to the east and west, and that ruminants are the primary species involved in virus maintenance. These species evidently serve as main amplifiers of infection during epizootics, but whether or not they also serve as sole virus reservoirs in inter-epizootic periods has yet to be determined.

INTRODUCTION

Rift Valley fever (RVF) is an arthropod-borne viral disease primarily affecting domestic animals with occasional human involvement (Daubney, Hudson & Garnham, 1931). Sheep and cattle are the main species affected and to a lesser extent goats. In these species the infection induces heavy economic losses due to high mortality in the young and abortions in pregnant animals (Easterday, 1965).

Natural disease has been known to occur only in Africa, mainly South, East, Central and lately North Africa. In several countries of sub-Saharan Africa where RVF as a clinical disease in domestic livestock has not been apparent, the existence of the disease has been based on serological evidence with some chance virus isolation as in Nigeria (Fagbami, Tomori & Kemp, 1973; Ezeifeka *et al.* 1982).

Zinga virus, first isolated in 1969 from *Mansonia africanus* mosquitoes in the Central African Republic (Digoutte *et al.* 1974), has recently been identified as RVF virus (Meegan *et al.* 1983; Georges *et al.* 1983). Isolation of this virus from mosquitoes and/or human infections in Senegal, the Central African Republic and Madagascar (Meegan *et al.* 1983) has further extended the range of RVF virus into West and Central Africa. The history and various other aspects of RVF have been comprehensively covered in a recent review by Shimshony & Barzilai (1983).

Before 1977 RVF was geographically limited to sub-Saharan Africa causing sporadic epizootics in animals and accidental contact infection in humans, which was rarely fatal. However, in 1977 and again in 1978 extensive epizootics of RVF with unprecedented human disease and fatalities occurred in Egypt (Abdel Wahab *et al.* 1978; Meegan, 1979; Laughlin *et al.* 1979). It was following its dramatic appearance in Egypt that RVF came into prominence as a potential international disease problem.

In the Sudan serological evidence of the existence of RVF was available in 1936, when a limited survey including parts of East and Central Africa revealed neutralizing antibodies in 6·7% of 164 human sera derived from Southern Sudan (Findlay, Stefanopoulo & MacCallum, 1936). The disease was of little or no concern until 1973, when RVF virus was identified for the first time as the cause of an extensive epizootic involving sheep, cattle and less severely goats in Kosti District on the White Nile, some 200 km south of Khartoum (Eisa & Obeid, 1977; Eisa, Obeid & El Sawi, 1977). Concurrently humans having contact with infected animals contracted a mild infection which eventually resolved spontaneously in about 2 weeks with no sequelae. In 1976 the disease again flared up still further north along the Nile Valley, first in southern parts of Khartoum Province and eventually in Khartoum North, where a small localized outbreak occurred in a Kuku dairy herd and involved attendants on the farm (Eisa *et al.* 1980). The disease has not been reported again since. The objective of this paper is to record the results of a preliminary survey using immunodiffusion, undertaken to assess the current status and significance of RVF in domestic animals of the Sudan.

MATERIALS AND METHODS

Precipitating antigen

The antigen used was a killed mouse liver preparation of the Egyptian 501 Zagazig strain of RVF virus (Meegan, 1979) extracted by the sucrose-acetone method of Clarke & Casals (1958) and inactivated with betapropiolactone. It was obtained in liquid and lyophilized forms with homologous sheep immune serum from the U.S. Navy Medical Research Unit No. 3, Cairo, Egypt. This antigen was the same preparation as that available for haemagglutination-inhibition (HI), complement-fixation (CF), immuno-fluorescent (IF) and some other tests. All stocks of antigen and antiserum were stored at −80 °C until required.

Serum samples

All sera were collected during 1979–83 from apparently healthy animals in various geographical regions. To eliminate passive immunity only adult animals were sampled, although no attempt was made to record the definite age, sex or general condition of donor animals.

Except for the samples from El Huda breeding centre all ovine samples were obtained from free-range sheep, as were the caprine and camel sera. On the other hand, all bovine sera except those originating from Jonglei, Kongor and Malakal of Upper Nile, Southern Region, were obtained from sedentary farm herds, including many Friesian and other crosses of high-producing animals. The camel sera were derived from slaughtered animals at Tambool market abattoir, Gezira

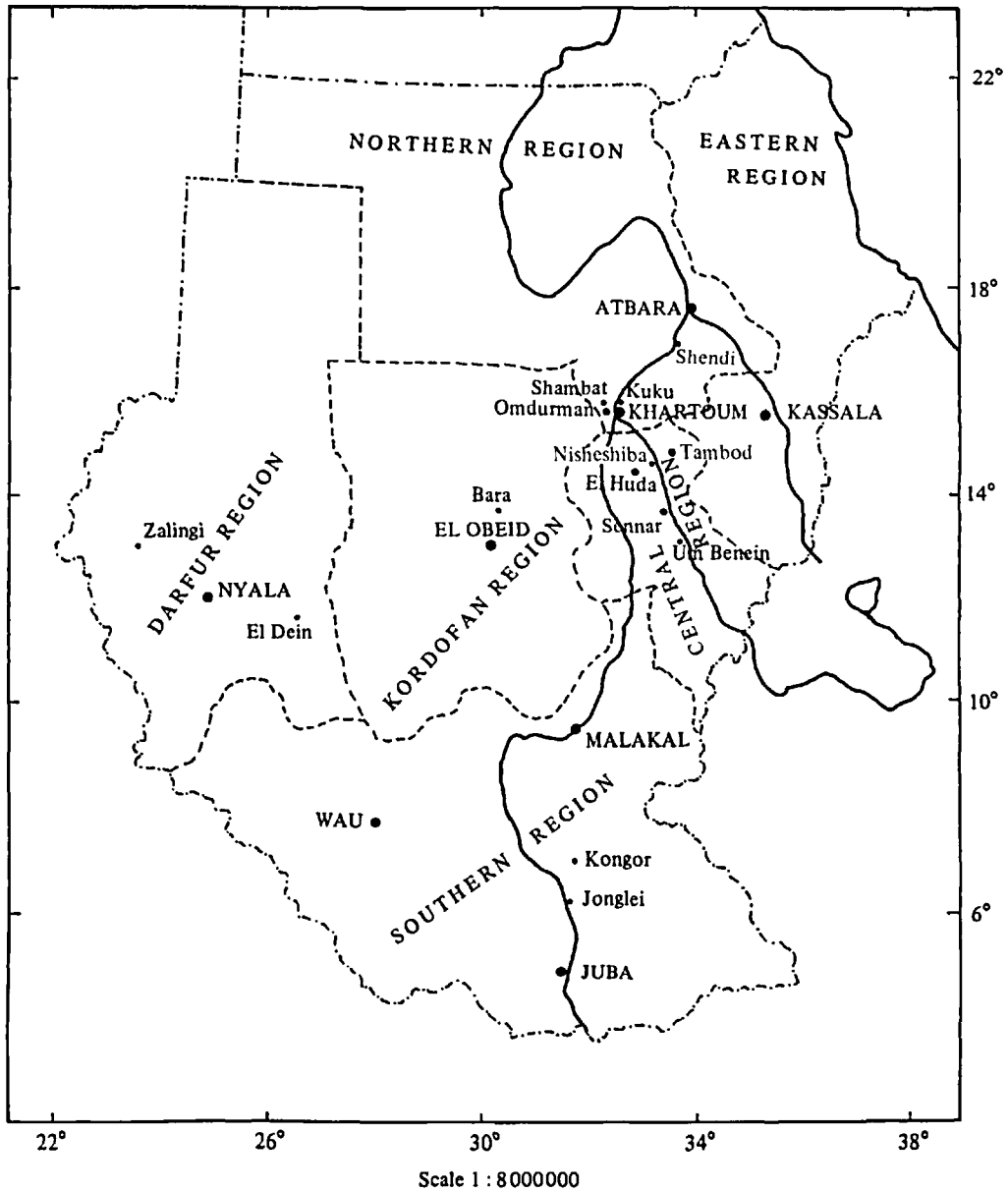

Fig. 1. Map of the Sudan, indicating origins and geographical locations of serum samples.

Province, Central Region and the equine sera from Nyala, Darfur Region and Atbara, Northern Region. The sheep and goats sampled were the indigenous desert-type long-tailed sheep and Nubian goats. Fig. 1 is a map of the Sudan showing the origins and geographical distribution of serum samples for easy reference.

Venous blood was obtained by venepuncture from live animals in sterile Vacutainer serum separation tubes (Becton-Dickinson & Co., Rutherford, New Jersey, U.S.A.). Blood from slaughtered animals was collected from the severed

https://doi.org/10.1017/S0022172400065207 Published online by Cambridge University Press

jugular vein. Blood samples were refrigerated overnight at 4 °C; the sera were separated by light centrifugation, decanted and stored at −20 °C until tested.

The agar-gel precipitation (AGP) test

Individual sera were examined for specific antibodies to RVF virus precipitating antigen. A modification of the technique described by Ouchterlony (1949) was adopted, essentially as outlined earlier (Eisa, Karrar & Abdel Rahim, 1979), except that the diffusion medium consisted of 1 % purified agar (Difco, Detroit, Michigan, U.S.A.) in buffered saline (pH 7·2). To check on lines of identity for interpretation, positive and negative control preparations were included in each series of tests. The plates were held at 25–27 °C in humidified air-tight chambers for 48–72 h before they were examined for precipitin lines. In the event of dubious or weak reactions, the test was repeated with refilling of the wells 2 or 3 times with intervals between the refills.

RESULTS

Positive homologous control systems produced clear precipitin lines of identity in 24–48 h. Many sera contained relatively little antibody and gave rise to precipitin lines closer to the wells containing the test sera. Only in rare instances was the line closer to the antigen-containing well. Where both antigen and test sera were weak, very faint or doubtful reactions were obtained. Final results were taken after the weak and doubtful reactions were duly rectified and precipitation patterns closely resembling lines of identity were scored positive.

The results of testing sheep and cattle sera are presented in Tables 1 and 2. Table 3 summarizes the results of testing goat, camel and equine sera. Of 376 ovine sera examined 128 (34·3 %) were positive, as were 67 of 202 (33·2 %) bovine, 27 of 123 (22 %) caprine, 3 of 38 (7·9 %) camel and 1 of 25 (4 %) donkey sera. All of 16 samples from horses were negative.

The percentage positive in sheep ranged from 20·5 in Atbara, Nile Province, Northern Region to 51·6 in Sennar, Blue Nile Province, Central Region. A comparably high figure of 50 % was found with sera from Juba District, Equatoria Province, Southern Region, Shendi of the Nile Province and Um Benein on the Blue Nile, Central Region. None of the samples from Bara, Northern Kordofan, Kordofan Region or Nyala, Southern Darfur Province, Darfur Region, both of Western Sudan, were positive.

Although the incidence of precipitating antibodies was generally high in cattle, it was notably higher among farm cattle compared to free-range animals. Thus the highest percentage positive (55 %) was detected in Um Benein breeding centre followed by Nisheshiba dairy (53·3 %) both of Central Region. By contrast, the lowest rates were recorded in free-range cattle from Kongor (10 %) and Malakal (13·6 %) of Upper Nile Province, Southern Region.

Of all the species examined the highest positive rate (73·3 %) was detected in goats from Wau, Bahr El Ghazal Province, Southern Region. Antibody-positive goats were also found in Atbara (30 %) and Kassala in Eastern Region (5·4 %). Again none of the samples from goats in Nyala, El Dein or Zalingi of Darfur Region in the west were positive.

Table 1. *Results of testing sheep sera for precipitating antibodies to RVF virus antigen*

Origin	No. of sera	No. positive	Positive (%)
El Huda Breeding Centre	34	15	44·1
Atbara	44	9	20·5
Shendi	56	28	50
Shambat	32	9	28·1
Sennar	62	32	51·6
Omdurman	89	22	24·7
Um Benein	10	5	50
Kassala	15	5	33·3
Juba	6	3	50
Bara	16	0	0
Nyala	12	0	0
Total	376	128	34·3

Table 2. *Results of testing cattle sera for precipitating antibodies to RVF virus antigen*

Origin	No. of sera	No. positive	Positive (%)
Jonglei	20	4	25
Kongor	20	2	10
Malakal	22	3	13·6
Shambat dairy	20	7	35
Atbara dairy	20	4	25
Um Benein Breeding Centre	20	11	55
Nisheshiba dairy	30	16	53·3
Kuku dairy	30	13	43·3
El Obeid dairy	20	7	35
Total	202	67	33·2

Table 3. *Results of testing goat, camel and equine sera for precipitating antibodies to RVF virus antigen*

Species	Origin	No. tested	No. positive	Positive (%)
Goats	Kassala	37	2	5·4
	Wau	30	22	73·3
	Atbara	10	3	30
	Nyala	14	0	0
	El Dein	16	0	0
	Zalingi	16	0	0
	Total	123	27	21·95
Camels	Tambool	38	3	7·9
Donkeys	Atbara	25	1	4
Horses	Nyala	16	0	0

DISCUSSION

Recent studies have shown that RVF virus is a phlebovirus (family Bunyaviridae) serologically related to viruses of the phlebotomus fever serogroup (Shope, Peters & Walker, 1980; Shope *et al.* 1981; Tesh, Peters & Meegan, 1981). The highest degree of cross-reactivity between RVF virus and phleboviruses is liable to occur

by the HI and IF tests, less by neutralization and least by the CF test (Shope *et al.* 1981). Although available experience is only limited, there is reason to believe that any of the basic techniques may be applicable for serological surveys of RVF provided the test chosen is properly performed and well controlled and interpreted. However, some tests, e.g. serum neutralization and plaque reduction have their limitations in that they use live virus. The AGP test has not been sufficiently investigated, although it is considered very specific, but like the CF test is not very sensitive (Swanepoel, 1981; WHO, 1982). Moreover the duration of persistence of the AGP antibody has not been as yet determined. However, despite its shortcomings the AGP test is simple to conduct, reliable when positive, and practical, and more importantly is a basic technique performed in many laboratories and requiring no sophisticated equipment. In this laboratory the AGP test has proved useful for serological identification of RVF virus isolates as well as for the detection of specific antibodies in animal sera. The test has also been satisfactorily applied for diagnosis of RVF in Zimbabwe (Swanepoel, 1981) and elsewhere for the detection of RVF antibodies in domestic animals (Ayoub & Allam, 1981; Ezeifeka *et al.* 1982).

Since no vaccination against RVF is practised in the Sudan, the results indicate that the infection is widely distributed in domestic ruminants as evidenced by the demonstration of specific AGP antibodies in appreciably high percentages of sera from sheep, cattle, goats and camels. In the absence of adequate information as to how long AGP antibodies persist postinfection and the test being a qualitative one, it could not be definitely established whether the detected antibodies were the result of a recent infection or one that had occurred in the distant past several years before sampling. However, taking into consideration the complete lack of reports of clinical disease recently, it appears more likely that these antibodies were due to a long-standing infection that might have continued quietly smouldering in animals and may eventually flare up in an outbreak or epizootic form should favourable conditions prevail.

The results indicate that sheep are the most frequently exposed to RVF virus followed by cattle and then goats and camels. Not surprisingly, the highest infection rate in sheep (51·6%) was recorded in Sennar District, which was affected by the 1973 epizootic (Eisa, Obeid & El Sawi, 1977) and it appears that the virus has persisted ever since in livestock in this area without causing a major epizootic or outbreak.

The relatively high incidence of infection in cattle may be attributed to the fact that the bulk of bovine samples was derived from sentinel farm cattle that invariably included significant proportions of the more vulnerable high-producing cross-bred animals. Moreover, it may be that due to faulty systems of management and hygiene farm cattle are more exposed to virus vectors than free-range animals.

The demonstration of antibodies in camels indicates that these animals are naturally infected but taking into account the complete lack of reports of clinical disease to date, it may be that RVF occurs in a subclinical or inapparent form in this species. However, naturally infected camels may present no signs of illness other than abortion (Scott *et al.* 1963). Hence the disease in camels is probably often overlooked.

The demonstration of antibodies in only 4% of donkeys and none in horses

suggests that RVF is insignificant in the equine species. Antibodies were detected as was RVF virus isolated from equines during the recent epizootics in Egypt (Darwish, Imam & Omar, 1978; Imam, El Karamani & Darwish, 1979). Nevertheless it is questionable whether equines have a role in the epizootiology of RVF, particularly since they have been shown to exhibit low levels of viraemia (Yedloutsching, Dardiri & Walker, 1981). According to these authors equines may rather serve as dead-end hosts during RVF epizootics and play little, if any role in the epizootiology of the disease.

A recent survey of Sudanese livestock (T. J. Bucci and colleagues, personal communication) demonstrated the presence of CF antibodies to RVF virus in 36·6 % of sheep, 22·2 % of camels, 5·4 % of donkeys, 2·5 % of goats and 1·6 % of cattle. Except for sheep and donkeys, the positive rates for which are generally comparable, these findings are widely different from those reported in this survey. Apart from possible differences in sampling systems, conditions and techniques, the inconsistency in results is difficult to explain in the absence of adequate knowledge of the relative dynamics of the various antibodies to RVF virus, although it is unlikely that the CF test would detect antibodies in a high percentage of animals infected more than two years (WHO, 1982).

The results obtained suggest that RVF is mainly prevalent in the rich savanna vegetation areas and woodlands of Southern Sudan as well as in the irrigated and well-watered agricultural areas close to the Nile Valley in the north. This conforms well with the so-far reported disease outbreaks and further suggests an association with insect vectors. It may thus be concluded that RVF virus is currently circulating in a longitudinal south–north range along the Nile Valley, with little or no extension to the drier acacia lands to the east and west.

It is noteworthy that in many of the areas where serological evidence indicated previous exposure, no reports of clinical disease have been made, which either means that overt disease had occurred but was overlooked, or that the infection is cryptically cycling in livestock but failing to assume epizootic proportions. It appears that a large proportion of livestock in these areas is immune due to previous exposure and the occurrence of a further epizootic will depend, in addition to other precipitating factors, on the replenishment of the existing livestock population by susceptible generations, which seems to contribute significantly to the spacing out of epizootics. However, in these areas crop-farming is generally more extensively practised, and livestock production, especially sheep and cattle, is of relatively less importance, except perhaps in certain areas of the south. As the bulk of these animals is predominantly concentrated in Western Sudan, where no evidence of RVF exists, resurgence of extensive epizootics remains a remote possibility, although small localized outbreaks may occur as happened in 1976 (Eisa *et al.* 1980). This probably also explains why no further epizootics have occurred since 1973.

It is finally concluded that ruminants are the main species involved in RVF virus maintenance in the Sudan, and there is little doubt that these species serve as the main amplifers of infection during epizootics. Yet whether they also serve as sole reservoirs of virus in interepizootic periods, or a natural reservoir exists in wild animals or rodents still remains an open question.

I am grateful to Dr O. L. Wood of the U.S. Navy Medical Research Unit No. 3, Cairo, for supply of antigen and antiserum and to the many veterinary colleagues in the field and central and regional laboratories who assisted in the collection of serum samples, and without whose co-operation this work would have been impossible. Due thanks are extended to Mr M. Z. Abd Alla for competent technical assistance, to Mr E. M. El Sheikh for typing the manuscript and to the Under-Secretary, Animal Resources, for permission to publish these results.

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