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# THE EXTENDED HORIZONS OF RIFT VALLEY FEVER: CURRENT AND PROJECTED IMMUNOGENS

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## HISTORY

The first detailed description of Rift Valley fever (RVF) was based on a sheep epizootic in an area where the great Rift Valley runs through Kenya (Daubney et al., 1931). RVF is a viral disease causing arthropod-borne epidemics of domestic animals during which man is also infected. Sheep epizootics resembling RVF occurred in Kenya during the first two decades of the 20th century, but it was not until 1930 that Daubney and coworkers studied the disease in detail and established the viral etiology of RVF. Initial scientific progress was rapid. Field observations and laboratory studies revealed that: (a) a wide variety of domestic, wild, and laboratory animals were susceptible to RVF virus infection with the characteristic pathological lesion being focal liver necrosis; (b) the virus could be isolated from, and transmitted by, a number of mosquito species; and (c) many African nations had serological evidence of human or animal infection by RVF virus (Easterday, 1965).

The disease continued to cause periodic epizootics, but until 1977 it was geographically limited to Sub-Saharan Africa. During many epizootics (and as a result of numerous laboratory infections), human RVF was described as a mild, dengue-like, febrile illness (Smithburn et al., 1949). However, during the 1975 epizootic in South Africa, severe clinical disease was reported in a small number of people, and the first fatalities directly attributable to RVF were documented (Van Velden et al., 1977). In 1977, an outbreak of the disease was reported in the Nile delta, a new geo-

graphic area and extensive human involvement with numerous fatalities occurred during the epizootic (Meegan, 1979).

The Egyptian epizootic re-emphasized the importance of this disease, as well as our lack of detailed understanding of the epidemiology, virology and pathogenesis of RVF. It also served as a graphic example of the potential of RVF to circulate in a number of differing geographic and climatic settings, since the virus has now spread in a 7,000-km north-south range throughout Africa. Although we do not know whether the virus will survive indefinitely in the Nile delta, there is reason to infer that it remains active.

## ETIOLOGY

The virus was originally classified as an ungrouped, arthropod-borne virus, with no serological relationship to other viruses. However, accumulating morphological and molecular evidence now indicates that RVF virus is similar to viruses in the family Bunyaviradae. Furthermore, Shope and Peters (1980) recently discovered that Rift Valley fever virus cross-reacts serologically with the phlebotomus fever group (Gordil, St. Floris, Punta Toro, Candiru, and perhaps others) by hemagglutination-inhibition tests. This antigenic cross-reactivity has been confirmed by indirect fluorescent antibody tests.

Electron micrographs of virions reveal a spherical viral particle, 90-100 nm in diameter, with apparent surface projections similar to other Bunyaviridae viruses (Murphy et al., 1973). The virion has a density of 1.18 g/ml in CsCl, and mild detergent treatment frees a nucleocapsid of density 1.29 g/ml. The nucleocapsid contains a 23,000-dalton molecular weight, nonglycosylated protein as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Two surface glycoproteins have been identified with apparent molecular weights of 65,000 and 70,000 daltons. The virion contains 3 unique RNA species (designated L, M, and S) with molecular weights by SDS-PAGE of 2.6, 1.1, and 0.7 x $10^6$ daltons (Rice et al., 1980). These properties are similar to those shared by the Bunyaviradae family (Obijeski and Murphy, 1977), and by extrapolation, the RNA of RVF virus will probably be found to be of single, negative strandedness, and associated with an RNA polymerase.

The presence of a segmented genome provides a mechanism for high-frequency genetic recombination or reassortment of the segments if a cell is infected with more than one virus. This phenomenon is used to engineer vaccines, and may well participate in the natural evolution of the myxovirus, influenza A. Two bunyaviruses, La Crosse and Snowshoe Hare, have been shown to exchange segments under laboratory circumstances (Gentsch et al., 1977), but the role of reassortment in the evolution of RVF or other bunyaviruses in nature is unknown.

The virus is sensitive to lipid solvents, can agglutinate sheep erythrocytes, and is inactivated quickly at low pH ($\leq$ 6.8). However, the virus is very resistant to temperatures below 60°C; it can be recovered readily from serum after several months storage at 4°C or 3 h at 56°C (Craig et al., 1967).

RVF virus readily grows in most commonly used cell cultures, except lymphoblastoid cell lines. Like other bunyaviruses, the virions form by budding from membranes of the endoplasmic reticulum and the Golgi apparatus, and are released by exocytosis and/or cell lysis. Plaques form readily under agar in many cell culture systems.

Although most field virus isolates are regarded as identical, Weinbren and coworkers (1957) made several isolations of an unusual variant of RVF in the Lunyo forest in Uganda. The Lunyo variant differs from classical RVF strains by being less pathogenic for adult mice upon peripheral inoculation, producing a more encephalitic clinical picture in adult mice, decreased stability in storage, and failure to hemagglutinate goose erythrocytes.

Recently, the prototype isolate from the Egyptian outbreak was compared to 1951 and 1975 South African isolates and a 1944 Ugandan isolate. They were indistinguishable by polyacrylamide gel electrophoresis of virion polypeptides (Rice et al., 1980), plaque reduction neutralization tests, or pathogenicity for 7 laboratory rodents. The Egyptian strain was 100,000-fold more pathogenic for the appropriate laboratory rat (Peters, unpublished data). Although enhanced pathogenicity for rats has been confirmed for other Egyptian isolates, the biological significance of this observation is unclear. Most of these same strains have been compared by oligonucleotide fingerprints, and were shown to

have many similarities, but each was readily distinguishable (unpublished data).

## CLINICAL DISEASE

There are few distinctive clinical features in RVF. Historically, illness in adult animals is characterized by a brief incubation period of 2 to 5 days, an abrupt onset, a sharp febrile reaction with temperatures as high as 42°C, a brief course of 2 to 4 days, followed usually by an uneventful recovery. Pregnant animals usually abort during the ensuing 2 weeks. Mortality in young animals, particularly lambs, is very high, whereas deaths in adult animals are less common. The most susceptible species are sheep, goats, and cattle, in that order. Pigs and horses are not clinically affected by the virus, although they may develop antibodies following exposure. Most of the pathologic lesions occur in the liver, which shows evenly distributed foci of necrosis with pin-point hemorrhages.

In man, the illness has also been thought of as nonspecific in its clinical signs. Incubation may range from 2 to 6 days, with a 2- to 5-day illness which is often biphasic; a prolonged convalescence is common. Symptoms may include headache, malaise, photophobia, epigastric tenderness, and nausea. Occasionally, an exudative retinal vasculitis occurs. If these lesions occur in the macula, severe visual impairment results and is permanent in many cases (Siam et al., 1980. Prior to the outbreaks of RVF in South Africa in 1975 and Egypt in 1977, fatal disease in man was considered to be an exceedingly rare event. Following these two major outbreaks, an increased respect for the human virulence potential of RVF has developed. Although denominators of infection are not known with accuracy, rates of mortality associated with fulminant hemorrhagic fever or encephalitis are variously estimated to be approximately 1% of reported RVF cases. There were 7 confirmed human deaths diagnosed as RVF in Africa during the 1974-75 epizootic in South Africa (Van Velden et al., 1977). The number of deaths associated with RVF-related encephalitis or fulminant hemorrhagic fever in Egypt is not known with certainty, but estimates have ranged from 60 to 600 during the 1977 transmission season. Whether high lethality for rats exhibited by the Egyptian isolates, as described above, correlates in any way with virulence in man is not known; the Egyptian epizootic produced

more human fatalities than any RVF outbreak previously recorded.

## EPIDEMIOLOGY

Evidence of RVF virus activity is based on serological evidence of (a) antibodies in humans and domestic animals, (b) the occasional isolation of virus from arthropods in the absence of overt disease in mammals, (c) the occurrence of small outbreaks without widespread transmission in animals or man, and (d) extensive epizootics, which may involve animals and humans over broad geographical areas. Based on this classification, a chronological list of recorded RVF activity is provided in Table 1. These data are extracted from the recent review by Peters and Meegan (1980).

The principal inference we may draw from this impressive record of RVF activity is that the virus is very widely disseminated throughout virtually all of Africa. It apparently exists in a diverse range of habitats and hosts. Evidence suggests that it may be transmitted by a great variety of biological and perhaps mechanical insect vectors.

### The Epizootic Cycle

There are records of at least 10 extensive epizootics of RVF in Africa during the last 50 years. These have occurred in Kenya, South Africa, Zimbabwe, Sudan, and Egypt. If we also consider the epizootics which have gone unrecorded and the enzootic activity that probably exists in West Africa, RVF must be considered a major factor in human and animal health in Africa.

We may infer from the sporadic nature of the major epizootics that a number of factors must coincide in order for widespread disease to occur. Verbal reports from South Africa and Zimbabwe describe the occurrence of very heavy, intermittent rains and large numbers of mosquitoes coinciding with the birth of lambs and calves in the spring, as the principal events that favor the epizootics of RVF. If these events further coincide with the existence of a sizable population of susceptible animals, the conditions for RVF activity would seem to be favorable.

TABLE 1. Chronology of Recorded Rift Valley Fever Activity in Africa

| Year | Area | Magnitude of Activity |
|---|---|---|
| 1912 | Kenya | Outbreak |
| 1930-31 | Kenya | Extensive epizootic |
| 1936-54 | Kenya | Limited outbreaks |
| 1936 | Mali, Gabon | Antibodies (man) |
| 1944-68 | Uganda | Virus isolations (mosquitoes, man) |
| 1950-51 | South Africa | Extensive epizootic |
| 1952-59 | South Africa | Limited outbreaks |
| 1954 | Rep. Congo | Antibodies (monkeys) |
| 1957-58 | Zimbabwe | Extensive epizootic (cattle) |
| 1959 | Nigeria | Virus isolation (sheep) |
| 1960 | Angola | Antibodies (man) |
| 1968-70 | Zimbabwe | Extensive epizootic |
| 1968 | Kenya | Extensive epizootic |
| 1968 | Uganda | Limited outbreak (man) |
| 1969 | South Africa | Extensive epizootic |
| 1969 | Chad | Antibodies (animals) |
| 1969 | Mozambique | Limited outbreak (cattle) |
| 1970-72 | Nigeria | Antibodies; isolates (Culicoides) |
| 1970-78 | Zimbabwe | Periodic isolates (mosquitoes, animals) |
| 1973 | Sudan | Extensive epizootic |
| 1974-75 | South Africa | Extensive epizootic |
| 1976 | Sudan | Outbreak |
| 1977-78 | Egypt | Extensive epizootic |
| 1978 | Zimbabwe | Extensive epizootic |

Perhaps the most interesting aspect of these acute observations of RVF activity in South Africa and Zimbabwe is the manner in which the virus is transmitted or disseminated during a major epizootic. These reports describe vertical, rather than horizontal or lateral, spread of the epizootic. That is, the virus does not appear to spread from one geographical area to another, horizontally or laterally. Rather, the dying calves or lambs and abortions occur everywhere simultaneously. Thus, it would appear that the virus already exists throughout the potential epizootic area, and that its spread or dissemination is vertical, i.e., from the enzootic, maintenance cycle, whatever it may be, to the epizootic, amplification cycle in the same geographical area.

Susceptible lambs and calves often have serum virus titers of $10^7$ to $10^9$; this is adequate to infect even the relatively inefficient mosquito vectors that exist in the South African high veldt. Mechanical transmission by mosquitoes and flies may be a factor also. Abortuses commonly attain virus titers of $10^9$/g of tissue, and this may represent a source for mechanical virus transmission by biting and nonbiting flies. By whatever mechanisms, cattle, sheep, and other ruminants appear to be major amplifying reservoirs; the young animals are particularly susceptible amplifiers of the virus.

## VECTORS

Approximately 17 species of mosquitoes have been implicated as possible RVF vectors, including Aedes tarsalis, Ae. de-boeri, Ae. aegypti, Ae. triseriatus, Ae. circumbuleolus, Ae. africanus, Ae. lineatopennis, Ae. dentatus, Ae. caballus, Culex theileri, C. neavei, C. pipiens, C. zombaensis, Eretmapodites quinquevittatus, E. chrysogaster, Mansonia africana, and Anopheles coustani (Peters and Meegan, 1980). In most countries, detailed studies have not been done on the prevalence of each species, population dynamics, feeding preferences, and efficiency as vectors of RVF virus. More extensive entomological investigations in South Africa strongly implicate C. theileri as the main RVF virus vector (McIntosh, 1972). In Egypt, C. pipiens appears to be the most prevalent species. RVF virus has been isolated from wild-caught C. pipiens and transmitted by a laboratory strain (Hoogstraal et al., 1979).

Recent data of McIntosh (personal communication) strongly suggest that mosquitoes are relatively inefficient vectors of RVF virus. High viremia levels were required to infect the mosquitoes tested, and low percentages of infected mosquitoes were able to transmit the virus to rodents or sheep. These are the only data we have found describing the vector efficiency of any insects for RVF virus; the conclusion we may draw is that only during periods of high vector activity in the presence of large numbers of highly susceptible animals potentially capable of circulating high titers of virus, is it possible for a major epizootic to occur.

There is little doubt that arthropods act as the main vector of the disease among animals. However, in addition

to biological transmission of the virus after replication in an arthropod, the unusually high RVF viremias in a number of hosts suggest that any hematophageous arthropod might mechanically transmit the virus. This possibility and the role of other arthropods, such as phlebotomine, Culicoides, and Simulium flies, requires more investigation.

## THE ENZOOTIC CYCLE

The most that can be said of the enzootic cycle of RVF is that it remains a mystery. The recent discovery that RVF virus is a member of the phlebotomus fever group of viruses suggests that the enzootic cycle may involve sandflies and certain, as yet unidentified, vertebrate reservoirs. Although we might initially be suspicious of rodents as enzootic reservoir hosts, the rodent survey by Swanepoel et al., (1978) in Zimbabwe showed little indication of RVF antibodies in the many rodents of the family Muridae that were tested. Rodent studies by McIntosh (1961) revealed rather low levels of viremia, i.e., levels that would appear inadequate to infect a high percentage of mosquitoes.

The low vector competence of the mosquitoes studied to date, the low viremias, and low antibody prevalence data in rodents and birds (Davies and Addy, 1979) leaves a significant gap in our knowledge of the enzootic cycle of RVF virus. Thus, we are unable to control or monitor the disease through the study of RVF in its reservoir habitat, because we know nothing about it. Nevertheless, in considering the varied range of virus activity in Africa, we must recognize the possibility that a variety of reservoir habitats may serve the needs of RVF virus for survival.

The unsettling conclusion we may draw from the available data on RVF epidemiology is that we may have no way of knowing if a particular area is already endemic. It is apparently not possible to recover RVF virus from the South African high veldt in the absence of an epizootic. When the appropriate rainfall pattern occurs, so as to favor the growth of large numbers of arthropods, occurring in conjunction with the presence of adequate numbers of susceptibles to serve as amplifiers, an enzootic occurs simultaneously at all points throughout the region. If this area is already seeded with the virus awaiting rainfall and susceptible animals, how do we know that other areas of the world are not similarly en-

demic? Might not the Sinai or the Negev or the uplands of southern Arizona in the United States be similarly propitious for an epizootic of RVF.

We might conclude that what the world needs is a good RVF vaccine.

## IMMUNOGENS

### Veterinary Vaccines

An inactivated vaccine for animal use exists and is used extensively in South Africa (Barnard and Botha, 1977). Two milliliters of this formalin-inactivated BHK-21 tissue culture supernatant with alum adjuvant administered to sheep protects against illness or viremia when they are challenged with RVF several months later, even though no significant serum antibody can be measured by virus dilution neutralization test. Cattle develop marginal neutralizing antibody responses and presumably would be protected also. This vaccine has been successfully used to interdict the spread of epidemic RVF in South African sheep; the duration of protection, fetal protection, colostral transfer of immunity, and the stability of the vaccine are uncertain.

Serial intracranial passage of the virus in mice results in attenuated RVF preparations, e.g., the Smithburn neurotropic strain. The Smithburn strain is highly immunogenic (Smithburn, 1949; Coakley et al., 1967) and has been used as an animal vaccine; it is abortogenic and thus is not entirely satisfactory. Further attempts at attenuation apparently led to loss of immunogenicity. RVF virus propagated repeatedly in tissue culture (Coakley et al., 1967) acquires similar properties to the high mouse-passage virus. These strains are potential immunogens for sheep and cattle, but are also highly reactogenic in field use. For the moment, there is no satisfactory attenuated RVF vaccine available for use to immunize animals safely and with a high expectation of immunogenic responses.

### Vaccines for Human Use

A safe and effective formalin-inactivated, human vaccine was developed by Randall and coworkers (1964). This vaccine is still investigational, but has been administered to seve-

ral hundred laboratory personnel with only occasional mild local reactions; its use has resulted in an impressive level of protection from illness. A few vaccinated persons have experienced asymptomatic rises in antibody titer while working with the virus. There have been no formal field tests, but several thousand doses have been used by at-risk laboratory and field personnel in endemic/epidemic areas, with no significant adverse effects reported, and apparent protection. Lyophilized vaccine has been stored at -20°C for as long as 10 years with no loss in potency. The major disadvantage with this product and, indeed, all inactivated RVF vaccines is the expense of production. Each milliliter of bulk product represents, at most, only a few doses; repeated inoculations are required for immunization. With an existing, effective inactivated vaccine, it seems unlikely that a live attenuated vaccine will be developed specifically for human use.

## The Next Vaccine

Although forecasts of future events are hazardous, we believe that the likelihood of further dissemination of RVF is high. The continuation of major epizootics in Africa is virtually certain. This mandates a requirement for a safe, effective, live attenuated vaccine suitable for immunizing hundreds of thousands, if not millions, of animals.

We believe the RVF vaccine that is needed should meet certain specifications or requirements:

1. It should be safe for use in susceptible animals of all ages.
2. It should be highly effective and confer a lifelong immunity from a single inoculation.
3. It should not be teratogenic or abortogenic.
4. It should be economical to produce, test, and control. Ideally, the bulk virus could be diluted 1,000 times or more for a final product. Thus, production would be relatively inexpensive.
5. The vaccine virus must not be transmissible from vaccinated animals to other animals, nor to arthropods.
6. The vaccine virus should not become latent, and recrudescence must never occur.
7. All developmental virus passages should be done in substrates that might ultimately permit the use of the virus in man, if tests proved it safe and efficacious.

Despite this formidable list of requirements, the projected vaccine may nevertheless be feasible. There are four possible developmental approaches that might be assessed. The first would be the classic method of obtaining an attenuated, naturally occurring virus clone from a virulent population of wild virus. The second approach involves the use of induced, high-level mutagenesis. This involves the deliberate induction of mutations in a virus population, the derivation of individual clones, and the assessment of each clone in terms of a virulence marker in vitro or in vivo. A third approach is the use of a closely related phlebotomus fever group virus that is infectious, but not virulent, for susceptible animals, and which will induce neutralizing antibodies and cross-protection against RVF. The fourth approach is to take advantage of the possibility of genetic reassortment and develop a new virus population consisting of reassorted segments from two or more related viruses.

No one could guarantee the success of such a projected effort, but we believe it is highly feasible. The initial work would have to begin in a high-containment laboratory and would require adherence to careful vaccine development technology. At the same time the most advanced techniques in viral genetics would be required for success.

In our opinion, this effort should receive the highest level of animal and human health research priorities. Only with a vaccine meeting the requirements specified can domestic ruminants be removed from the potential amplification reservoir for Rift Valley fever virus.

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