Molecular Epidemiology and Emergence of Rift Valley Fever

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Rift Valley fever (RVF) is a mosquito-borne viral disease which manifested itself during recent epidemics and revealed its significant potential of emergence. Studies on molecular epidemiology undertaken to better understand the factors leading to RVF emergence, have confirmed the mode of circulation of the virus and highlithted probable risks and obstacles for prevention and control. As for several other viral agents, molecular epidemiology is becoming a useful tool in the study of the emergence of RVF as a serious infectious disease.

Key words: arboviruses - Rift Valley fever - molecular epidemiology - emergence - phylogeny

Rift Valley fever (RVF) is an arboviral disease transmitted by mosquitoes in Africa. RVF affects primarily ruminants causing high mortality in offspring and abortions in pregnants females and occasionally humans, whose infection leads to a clinical picture which ranges from a mild febrile case to hemorragic fever with complications such as hepatitis, encephalitis and retinitis (Laughlin et al. 1979). In 1977, a severe outbreak of RVF occurred in human and livestock populations of Egypt (Meegan 1981). Although RVF was known for more than 40 years at that time, the extensive morbidity and mortality observed in humans appeared as a novelty in the history of this disease, therefore, emphasizing RVF as a serious emerging threat for humans and animals health. Futher large scale epidemics in Mauritania (Digoutte & Peters 1989), Madagascar (Morvan et al. 1991, 1992a, b), Egypt (Arthur et al. 1993) and very recently in eastern Africa (Anonymous 1998) confirmed the major impact of RVF on public health through its continuing emergence. Thus, RVF constitutes an excellent model to overview factors involved in arboviruses emergence because most of the concepts relative to emerging diseases may be illustrated along its natural history.

Control of RVF implies the better identification of factors involved in its emergence and its maintenance in nature. It is also necessary to understand the rules and modalities of circulation and evolution of RVF virus (RVFV) in Africa. These latter objectives have been addressed by studying the variability among RVFV isolates by serological (Besselar et al. 1991) and molecular methods (Battles & Dalrymple 1988, Sall et al. 1997a, b). This paper aims the discussion of some of the aspects and contributions of molecular epidemiology towards the elucidation of RVF emergence.

BACKGROUND

Discovery of RVFV and recent major epidemic/epizootics

RVFV was first isolated in 1930 near lake Naivasha in Kenya by Daubney et al. (1931). Since then, the virus has been shown to be widespread in subsaharian Africa and in Egypt (Meegan & Bailey 1989). Major epidemic/epizootics occurred in Egypt in 1977 (200,000 humans infections and 600 deaths) and in 1993, Mauritania in 1987 (200 human deaths), Madagascar in 1991 and in eastern Africa (89,000 infections and more than 500 deaths reported so far) with the last recent outbreak in 1997-1998 in Kenya, Tanzania, Somalia.

The etiological agent of RVF

RVFV is a member of Bunyaviridae family, *Phlebovirus* genus (Murphy et al. 1995). Its genome consists in three negative single stranded RNA segments referred as L, M and S respectively for large, medium and small. The L segment codes for the L protein which is the viral polymerase. The M segment codes for glycoproteins G1 and G2 and two others proteins of 78 and 14 K. The S segment codes for the nucleoprotein N and the non structural NSs protein using an ambisense strategy (Bouloy 1991, Elliott et al. 1991, Giorgi 1996, Schmaljohn 1996).

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Epidemiology of RVF

The epidemiology of RVF consists in both epizootic and interepizootic cycles (Meegan & Bailey 1989). Epizootics of RVF in Africa occured often when unusually heavy rainfall were observed. During an epizootic, virus circulates among infected arthropod vectors and mammalian hosts, particularly cattle and sheep, which represent the most significant livestock amplifiers of RVFV. The inter-epizootic survival of RVFV is believed to depend on transovarial transmission of virus in floodwater *Aedes* mosquitoes (Linthicum et al. 1985). Virus can persist in mosquitoes eggs until the next period of heavy rainfall when they hatch and yield RVFV infected mosquitoes. Depending on factors such as availability of sufficient numbers of competent mosquito vectors, presence of susceptible vertebrates, appropriate environmental conditions, infected mosquitoes have the potential to infect a relatively small number of vertebrate hosts or to initiate a widespread RVF epizootic.

Control and prevention of RVF

Vaccines have been the principal mean used to control RVF. Two types of vaccines have been described for use against RVF: inactivated and live-attenuated.

Formalin-inactivated RVF vaccines have been used to immunize animals, laboratory workers, veterinarians and other people at high risk of exposure to RVFV. The cost of the vaccine, the requirement for multiple inoculations and the time interval required to mount a protective immune response, all limit its use for veterinary purposes.

Two live attenuated vaccines, the Smithburn vaccine, also referred as Smithburn neurotropic strain or SNS (Smithburn et al. 1949), and MP12 (Caplen et al. 1985) have been developped. The Smithburn strain is the only widely available veterinary vaccine but has serious limitations in practical use, because it has been proven to be teratogenic, cause abortions and encephalitis in young lambs.

Possessing attenuation markers in all three segments, MP12 has a very low probability of reversion (Saluzzo & Smith 1990, Vialat et al. 1997) and has been inoculated into more than 100 people and shown to be safe and immunogenic (Peters 1997). MP12 was also promising in laboratory trials in domestic animals (Morrill et al. 1987, Morril & Mc Clain 1996), but vaccination of pregnant ewes revealed that the virus caused teratogenic effect if inoculated during the first trimester of pregnancy (Erasmus and Bishop, pers. commun.). Another attenuated virus, clone 13, a naturally attenuated strain, is very promising regarding the preliminary results obtained in terms of immunogenicity and safety (Muller et al. 1995).

MOLECULAR EPIDEMIOLOGY OF RVFV

Investigation on the variation among RVFV isolates using serological tests based on the antigenicity of structural proteins (Saluzzo et al. 1989a,b, Besselar et al. 1991) or genetic methods such as T1-oligonucleotide fingerprints (Peters & Linticum 1994) and, more recently sequencing, (Battles & Dalrymple 1988) indicated only minor variations among RVFV natural isolates. To further analyze the genetic diversity of RVFV (Sall et al. 1997b), we selected a panel of 18 strains (Table I) isolated over some 50 years from various hosts and geographical origins and we sequenced directly their NSs coding region on the S segment after a step of reverse transcription-polymerase chain reaction amplification (RT/PCR). A 50% majority rule consensus tree derived from the sequences analyzed are presented in Fig. 1. The NSs coding region sequences clustered in three major lineages supported by high bootstrapping values and by using different phylogenetic inference procedures (e.g., maximum likelihood, parsimony and distance methods) and correlated with the geographic origin of the isolates and are referred as West Africa, East-Central Africa and Egypt. While the West Africa group was homogenous with strains from Mauritania, Senegal, Guinea and Burkina Faso, the East-Central Africa and Egypt ones appeared to be heterogenous.

As expected, the Egyptian group contains strains isolated in 1977 and 1993 epidemics, which appear in the phylogeny as sister groups suggesting that either the virus remained endemic between the two outbreaks or have been reintroduced in 1993 from the same source (probably Sudan) as in 1977. To explain the reemergence of RVF in Egypt after years of silence despite intensive surveillance, Peters (1997) proposed that the virus was reintroduced through an incompletely inactivated RVF veterinary vaccine. Furthermore, Ar MAD 79, which is the first isolation of RVFV in Madagascar, clustered in Egypt group and then confirmed data obtained by Morvan et al. (1991) who analyzed the antigenic properties of the N protein.

Secondly, the East-Central African group clustered isolates from Uganda, Central African Republic, Mauritania and Senegal. The presence of An MAD 91 in that group suggested that this latter strain was probably introduced in Madagascar from the eastern coast of Africa. This latter assumption also implies that there was at least two introductions of the virus in Madagascar but also several lineages coexist in East Africa. Moreover, in

TABLE I

Characteristics of the Rift Valley fever virus isolates analyzed by sequencing

Code	Strain	Year of isolation	Origin	Source
SNS	Smithburn	1944	Uganda	Entebbe strain
Ar UG 55	Lunyo	1955	Uganda	Mosquito
Ar RCA 69	Ar B 1976	1969	CAR	Mosquito
H EGY 77	ZH 548	1977	Egypt	Human
MP 12[a]	MP12	1985	Egypt	ZH548 strain
Ar MAD 79	Ar Mg 811	1979	Madagascar	Mosquito
Ar SEN 84	Ar D 38661	1984	Senegal	Mosquito
An GUI 84	An K 6087	1984	Guinea	Bat
Ar BUF 84	Ar D 38457	1984	Burkina Faso	Mosquito
H1 MAU 87	H D 47502	1987	Mauritania	Human
H2 MAU 87	H D 47311	1987	Mauritania	Human
H3 MAU 87	H D 47408	1987	Mauritania	Human
H4 MAU 87	H D 48255	1987	Mauritania	Human
An MAD 91	An Mg 990	1991	Madagascar	Bovine
Ar SEN 93	Ar D 104769	1993	Senegal	Mosquito
An SEN 93	An D 106417	1993	Senegal	Zebu
B EGY 93	B EGY 93	1993	Egypt	Buffalo
H EGY 93	H EGY 93	1993	Egypt	Human

a: laboratory-attenuated strain derived from a wild strain; SNS: Smithburn neurotropic strain; H: human; Ar: arthropode; An: animal; B: buffalo.

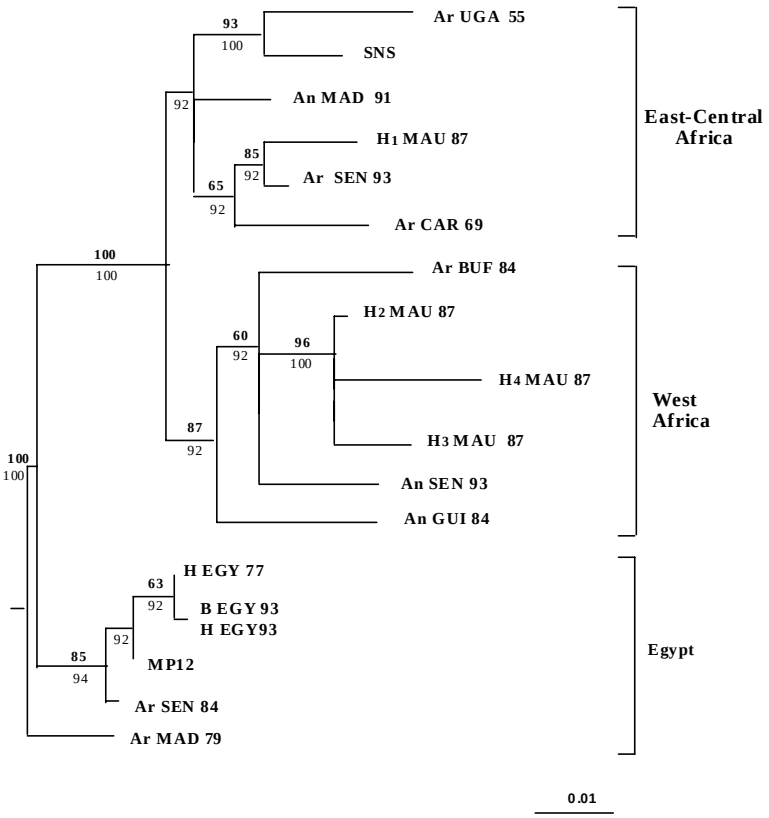

Fig. 1: phylogenetic tree for the NSs gene of several Rift Valley fever virus isolates. Values above branches indicate the level (%) of bootstrap support using maximum parsimony after 500 iterations. Values below branches indicate the number of times a given node was observed on a majority rule consensus of 50 trees with equivalent likelihood (LnL). Branch lengths are shown proportional to the number of substitutions per 100 residues. The rooting shown here was determined by the inclusion of the SSF NSs sequence.

Uganda, despite 11 years separating the isolations of Ar UGA 55 and Entebbe strain, the parental strain of Smithburn vaccinal strain, RVFV did not show much genetic diversity, suggesting a maintenance mechanism through an endemic/enzootic cycle, possibly involving comparatively little viral activity, since increased genetic diversity for a given mutation rate entails an increase on the effective viral population size. Surprisingly, H1 MAU 87 and Ar SEN 93 belonged to the East-Central Africa group.

The West Africa group appeared to be homogenous and suggested circulation of similar variants in Senegal, Mauritania, Guinea and Burkina Faso. It is noteworthy that H2, 3, and 4 MAU 87 clustered near each other and were isolated from fatal cases whereas H1 MAU 87 which was isolated from a febrile case clustered together with Ar SEN 93 unexpectedly in the East-Central Africa lineage. Moreover, one may deduced, from the strains distribution on Fig. 1, that there are two areas of circulation of RVFV in Senegal: (i) the Northern Sahelian zone where Ar SEN 93 and H1 MAU 87 were isolated and, (ii) the Sudano-Guinean zone where An SEN 93 was isolated.

Groupings of Ar SEN 84 with Egyptian strains on one hand and H1MAU 87 and Ar SEN 93 with eastern and central African strains on the other hand, were quite unexpected and led us to hypothesize genetic exchange through reassortment to explain these puzzling clusterings. In order to check this hypothesis further sequencing and phylogenetic analysis were undertaken both on L and M segments. Although, this hypothesis is still under investigation, one may obviously speculate by anticipation that such a mechanism *in natura* would have important implications on epidemiology and emergence of RVF in Africa (see below).

EMERGENCE OF RVFV

Various factors contributing to the emergence of infectious diseases were classified by Lederberg et al. (1992) and analyzed from the point of view of RVF by Wilson (1994) and summarized in Table II. Emergence of RVF was also discussed by Peters (1997) with special reference to Madagascar, distant spread of the virus to Egypt and historical speculations. These two papers emphasized the multifactorial aspect of RVF emergence and the central role of water and ecological change as factors triggering epidemics. Water is usually involved either through dams or irrigation for the sake of agriculture development, as illustrated by Egypt in 1977 and Mauritania in 1987 or, under excessive rainfall and floodings as observed during the 1997-98 outbreak in eastern Africa. Concerning the impact of ecological changes as deforestation and agricultural pratices change, the outbreak in Madagascar in 1991 has been shown to be a very instructive example (Peters & Linthicum 1994, Peters 1997).

TABLE II

Summary of Rift Valley fever (RVF) emergence factors described and analyzed by Wilson (1994)

Factors of emergence	Examples relative to RVF
Economic development/ Land use	Dams and irrigation, pasturage improvement
Human demography and behavior	Living with domestic ungulates, slaughter of sick animal, Vaccination of healthy animals
International travel and commerce	Domestic ungulates export, human travel and migration
Biological adaptation and change	Increased viral virulence, improved vector competence, greater animal susceptibility
Climate events	Excessive rainfall

Although these two key factors were clearly identified and characterized, data derived from molecular epidemiology are needed for a comprehensive view of RVF and its emergence process. Our work, although still preliminary allowed to illustrate the contribution of molecular epidemiology for, (i) the understanding of two modes of circulation of viral strains and (ii) delineation of genetic aspects of the virus, which may turn out to become potential obstacles for the prevention and control of the disease.

Modes of circulation of RVFV

Regarding the molecular epidemiology data about RVFV, two modes of circulation may be illustrated (Fig. 2): (i) distant spread from one region to another and (ii) local circulation in an enzootic/endemic area.

Distant spread was illustrated by introduction of RVFV in Egypt (1977) and Madagascar (1979 and 1991) probably from eastern or central Africa (see molecular epidemiology). It is interesting to emphasize that in both cases, possibly an antigenically and phylogenetically "new" virus was introduced in an area exempt of RVFV, raising the issue about the role of herd immunity for both, humans and animals populations, as a factor of emergence of the virus.

Concerning local circulation in an enzootic/endemic area, Senegal is an instructive example

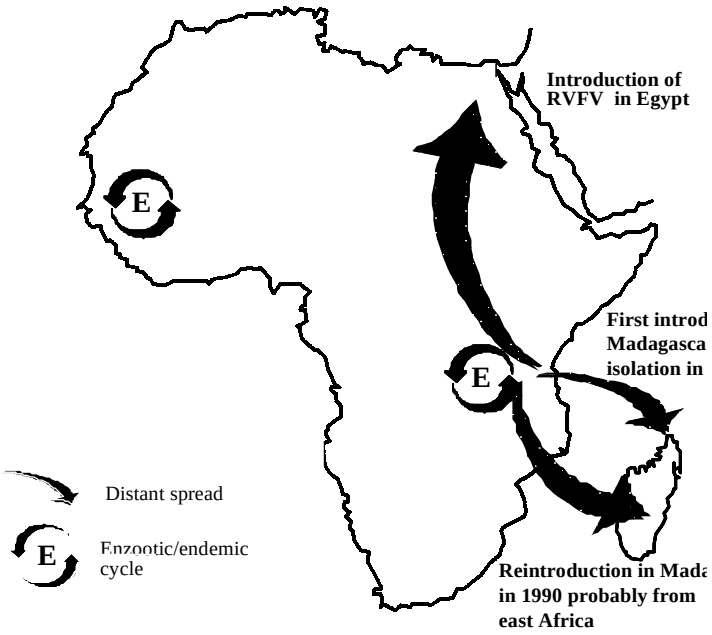

Fig. 2: possible modes of circulation of Rift Valley fever.

because it showed reemergence of the virus from a pool of existing enzootic/endemic strains under a similar process although the ecological context of transmission is different between the north and the south of that country. As far as RVF is concerned, Senegal can be divided in two areas (Sall et al. 1997b) from which the virus have emerged as demonstrated by isolations in 1993 (Zeller et al. 1997): (i) the Sahelian zone, where southern Mauritanian and northern Senegalese strains are circulating and, (ii) the Sudano-Guinean zone where southern Senegalese strains are in contact with those from bordering countries.

Prevention and control

In the field of prevention and control of RVFV, molecular epidemiology studies highlighted a potential major obstacle to the use of live attenuated vaccines. Indeed, the possibility of the existence of reassortment in nature raised by the unexpected groupings (Ar SEN 84, H1 MAU 87 and Ar SEN 93) would emphasize the risk of generating uncontrolled chimeric viruses.

CONCLUSION

Although molecular epidemiology has been shown to be informative for a better understanding on different facets of RVFV emergence, many questions such as those relative to the sylvatic cycle of the virus for instance remain unanswered. Meanwhile, surveillance of RVF and awareness should be improved and reinforced since it is so far the only conceivable way to prevent RVFV emergence with its toll of deaths, sickness and economic loss.

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