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ANTIBODY PREVALENCE AGAINST HAEMORRHAGIC FEVER VIRUSES IN RANDOMIZED REPRESENTATIVE CENTRAL AFRICAN POPULATIONS

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SUMMARY

Between 1985 and 1987, 5,070 randomly selected persons living in 6 central African countries (Cameroon, Central African Republic, Chad, Congo, Equatorial Guinea and Gabon) were checked for serological evidence of haemorrhagic fever. Rural and urban areas were studied, including ecoclimatic zones ranging from dry savana to tropical rain forest. Virus-reactive antibodies were found with all antigens tested, and the global prevalence of positive sera was distributed as follows: Crimean-Congo haemorrhagic fever virus, 0.22 %; Rift Valley fever virus, 0.18 %; Ebola virus, 12.40 %; Marburg virus, 0.39 %; Lassa virus, 0.06 %; and Hantaan virus, 6.15 %. A significant variation in antibody prevalence was observed within the study regions. Association between the viruses was not observed.

KEY-WORDS: Haemorrhagic fever; Seroprevalence, Central Africa.

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INTRODUCTION

Haemorrhagic fever viruses (HFV) are highly pathogenic agents capable of causing severe epidemic disease with high mortality and morbidity. Neither an effective specific treatment nor a preventive vaccine are available for most of these diseases. They represent a potential public health threat through the importation of causative agents into non-endemic areas by infected travellers. In many cases, these viruses pose significant hazards to clinical and laboratory personnel.

Since 1985, the Organisation de coordination pour la lutte contre les grandes endémies en Afrique centrale (OCEAC) and the concerned national health departments have participated in a viral haemorrhagic fever (VHF) surveillance program to evaluate the frequency and distribution of VHF in endemic areas of Africa. Hospital clinics were surveyed to identify haemorrhagic diseases of unknown origin and serosurveys were conducted to establish antibody prevalence rates and define populations at risk. The seroepidemiological sample survey was used for VHF, human immunodeficiency virus type 1 and hepatitis B antibody prevalence studies (Merlin *et al.*, 1987). Serological surveys took place in 14 areas, within different ecosystems, of the following countries of central Africa: Cameroon, Central African Republic (CAR), Chad, Congo, Equatorial Guinea and Gabon.

MATERIALS AND METHODS

The sampling method and laboratory procedures have been previously reported in detail (Gonzalez *et al.*, 1983; Merlin *et al.*, 1987) and are briefly described.

Populations investigated.

From January 1985 to June 1987, 5,070 randomly selected persons were investigated. The sampling method was derived from one used by Henderson and Sundaresan for evaluating vaccinal coverage of the extended programme of immunization (Henderson and Sundaresan, 1982). To evaluate the antibody prevalence in the study population, a sample size of 300 to 400 persons, divided into 30 randomly selected clusters, provided statistical reliability and were representative, within each sample, in accordance with the sex and age distribution of the normal population of each area investigated. After obtaining the individual's consent, subjects were bled either

CAR = Central African Republic.
CCHF = Crimean-Congo haemorrhagic fever.
IFA = immunofluorescent assay.
HFRS = haemorrhagic fever with renal syndrome.
HFV = haemorrhagic fever virus.
OCEAC = organisation de coordination pour la lutte contre les grandes endémies en Afrique centrale.
RVF = Rift Valley fever.
VHF = viral haemorrhagic fever.

by "Vacutainer" at the venous brachial plexus or by finger puncture using the "Microtainer" system (Becton Dickinson). The participants name, adress, sex and age were recorded.

Geographical areas.

Numerous climatic and ecological variables have been used to describe African environments: rainfall and representative plant types have been the most common factors (White, 1980; Boulvert, 1986; Gonzalez, 1986). Variables such as animal species have been used but the susceptibility to annual fluctuation makes them less reliable indicators of the physical environment. Using annual rainfall and characteristic vegetation, the environments of 6 participating African nations were separated into phytogeographic zones that were ecologically uniform (table I, fig. 1). A total of 14 accessible survey districts were selected from 5 strata. The names assigned to study regions corresponded to the names of major villages situated within the study area.

Samples and laboratory method.

A classical indirect immunofluorescent assay (IFA) was used to test each serum specimen for VHF-reactive antibody (Wulff and Lange, 1975). Sera were diluted 1/16 in phosphate-buffered saline and screened for IFA reactivity using polyvalent spot slides consisting of equivalent numbers of inactivated Crimean-Congo haemorrhagic fever (CCHF) virus (10200 strain), Rift Valley fever (RVF) virus (ZH 501 strain), Lassa virus (Josiah strain), Ebola virus (with the reference strains of Mayinga from Zaire and Boniface from Sudan), Marburg-virus-(Musoki strain)-infected Vero cells and fluorescein-conjugated anti-human immunoglobulins (Johnson *et al.*, 1981).

TABLE I. — **Area studied for HFV antibody prevalence in central Africa.**

Country	District	Phytogeographical domain	Census
Cameroon	Mora	Sahelian	160,000
	Maroua	Sudano-Sahelian	100,000
	Nkongsamba	Sudano-Guinean	85,000
CAR	Bangui	Sudano-Guinean	400,000
Chad	N'Djamena	Sahelian	450,000
Congo	Pointe Noire	Congo-Guinean	350,000
	Brazzaville	Guinean-Congolese	300,000
Equat. Guinea	Bioco Island	Congo-Guinean	50,000
	Nsork	Congo-Guinean	9,500
Gabon	Libreville	Congo-Guinean	180,000
	Port-Gentil	Congo-Guinean	85,000
	Ogooué Ivindo and Haut Ogooué	Congo-Guinean	70,000
	Ngounié	Guinean-Congolese	15,000
Total	—	—	2.254,000

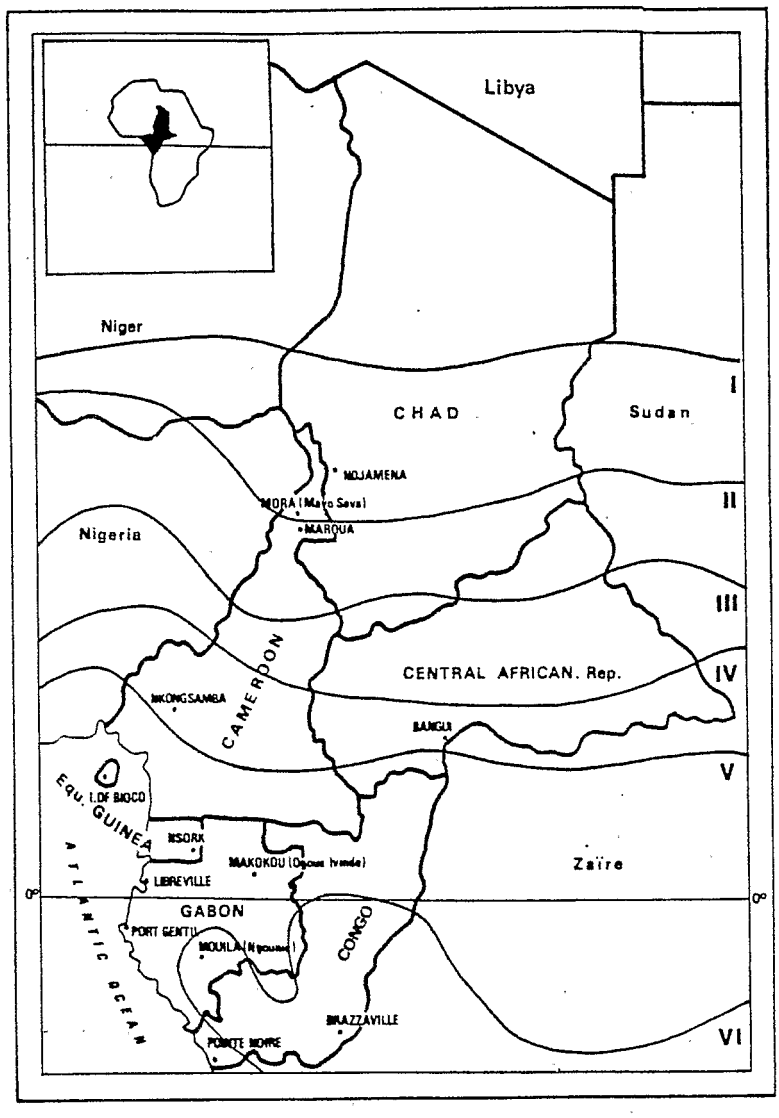

FIG. 1. — *Geographical distribution of the area studied: political and phytogeographical zones.*

Phytogeographical domains: I = Sahelian; II = Sudano-Sahelian; III = Medio-Sudanian; IV = Sudano-Guinean; V = Congo-Guinean; VI = Guinean-Congolese/Zambezian transitional.

Antibody titres were determined by titrating seropositive samples on inactivated monovalent spot slides consisting of Vero cells infected with a single HFV strain. (Polyvalent and monovalent spot slides were kindly provided by the Disease Assessment Division, USAMRIID, Fort Detrick, USA). Seroreactivity to Hantaan virus, the causative agent of haemorrhagic fever with renal syndrome (HFRS) was determined using monospecific Hantaan-virus-(Prototype 76118 strain)-infected Vero cell spot slides; provided by Dr James Leduc of the United States Army Medical Research Institute of Infectious Diseases (Gonzalez *et al.*, 1983, Meunier *et al.*, 1987a).

RESULTS AND DISCUSSION

For each location, we determined that the age and sex distribution of our samples did not differ significantly from that of the population censuses.

The specificity, sensitivity and limitations of the IFA test have been demonstrated and previously reported. IFA is highly specific with limited or absent cross-reactivity (RVF, Lassa, CCHF) with other related viruses present in central Africa (Gonzalez *et al.*, 1983; Peters and Leduc, 1987). The specificity of tests for Marburg, Ebola and HFRS is discussed below. We found no significant differences among age groups or between sexes in antibody prevalence against each antigen tested, with the exception of Ebola virus in one geographic location (discussed below).

CCHF.

Of 11 positive samples (titres ranging from 1/16 to 1/64), 7 were from coastal areas, Pointe Noire and Bioco Island (table II). The 2 sera from Cameroon positive to CCHF (1/32 and 1/64) were also positive against Ebola antigen.

CCHF human cases, virus isolates and antibody serosurveys have revealed widely distributed clusters of virus activity in the old world (Watts *et al.*, 1988). In our study, antibody prevalence (0.22 %) was about the same as that observed in other human populations in Africa (Gonzalez *et al.*, 1983; Georges and Gonzalez, 1986; Meunier *et al.*, 1987a; Meunier *et al.*, 1987b). Our results showed a scattered distribution of the virus from the Sahel to the rain forest and beyond its known range into Cameroon and Equatorial Guinea.

A major vector, *Hyalomma marginatum rufipes,* is present in Cameroon, CAR and Chad, but has not been described in the Congo or Equatorial Guinea (Morel, 1969). Despite the low antibody prevalence in Mora (0.5 %), the present observations suggest an enzootic situation in north Cameroon, where cattle and sheep are abundant and may represent a human CCHF biohazard (Georges and Gonzalez, 1986). In Congo and Equatorial Guinea, where vectors have not been identified, the human cases may represent travellers to endemic areas or cases of infection from contaminated imported domestic ruminants.

TABLE II. — **Prevalence rate of antibody against HFV in people from central Africa randomly selected during 1985-1987.**

Locality	Sample size	% (nb positive) CCHF	RVF	EBO	MBG	LAS	HAN
I — N'Djamena	334	0.0	0.30 (1)	3.59 (12)	0.30 (1)	0.06	1.19 (4)
Mora	395	0.50 (2)	0.25 (1)	10.88 (43)	0.0	0.50 (2)	NT
II — Maroua	379	0.0	0.0	10.29 (39)	0.0	0.0	NT
IV — Nkongsamba	378	0.0	1.05 (4)	1.85 (7)	0.0	0.0	0.79 (3)
Bangui	327	0.0	0.0	32.72 (107)	0.0	0.0	NT
V — Bioco Island	308	1.29 (4)	0.0	16.23 (50)	2.59 (8)	0.32 (1)	NT
Pointe Noire	360	1.11 (4)	0.0	7.77 (28)	3.05 (11)	0.0 (3)	NT
Port Gentil	376	0.0	0.53 (2)	7.44 (28)	0.0	0.0	8.30 (25) (*)
Nsork	380	0.26 (1)	0.26 (1)	16.05 (61)	0.0	0.0	14.21 (54)
Libreville	349	0.0	0.0	12.03 (42)	0.0	0.0	8.30 (29)
Makokou (Ogoué Ivindo)	360	0.0	0.0	21.66 (78)	0.0	0.0	0.0
Franceville (Haut Ogoué)	384	0.0	0.0	14.58 (56)	0.0	0.0	6.92 (29) (**)
VI — Brazzaville	368	0.0	0.0	6.25 (23)	0.0	0.0	NT
Mouila (Ngounié)	372	0.0	0.0	14.78 (55)	0.0	0.0	9.13 (34)
Total	5,070	0.22 (11)	0.18 (9)	12.40 (629)	0.39 (20)	0.06 (3)	6.15 (178) (***)

Roman numeral = phytogeographical zone numbering, refer to fig. 1.
The relative precision of seroprevalence rates observed in the 14 random ranges varies from 25 to 55 % according to a high or low frequency of positivity.
EBO = Ebola; MBG = Marburg; LAS = Lassa; HAN = Hantaan; NT = not tested.
(*) 301 sera tested.
(**) 419 sera tested.
(***) 2,893 sera tested.

RVF.

One third of the study areas had low antibody prevalences against RVF virus (0 to 1.05 %), whereas 4 of the 9 positive specimens were found in the village of Nkongsamba (Cameroon) and antibody titers ranged from 1/16 to 1/128.

Nkongsamba is one of the most important points at which cattle are gathered before being sent to the capital Yaoundé. Two other positive areas, Mora and N'Djamena, are situated in a typical Sahelian domain where RVF virus is active, relative to a large population of herders (Jouan *et al.*, 1988). In 1987, a severe non-fatal human case of RVF was identified in Pete (35 km north of Maroua), a village in the major livestock raising area of north Cameroon (J.P. Durand, pers. comm.).

Nevertheless, antibody prevalence remained low (0.18 %), comparable to that observed for areas without epizootics where the rate of seropositivity is generally less than 15 % (Peters and Leduc, 1987). The positive areas of Nsork and Port Gentil are situated in a Congo-Guinean domain, analogous to the environment of central Africa where sporadic human cases are observed in cattle-raising areas. Nevertheless, while RVF is enzootic in central Africa, human infection appears to be rare and no epidemic has yet been recognized (Gonzalez *et al.*, 1987).

Ebola and Marburg virus diseases.

Since the discovery of Marburg and Ebola viruses, IFA and complement fixation have been used systematically for serosurvey and serodiagnosis throughout Africa (Bergmann and Bourree, 1982, Gonzalez *et al.*, 1983; Johnson *et al.*, 1983). Despite the high fluorescent antibody prevalence without identified VHF cases, in the absence of cross-reactivity with other known viruses, IFA has been kept as a reference test.

The highest seroprevalence against investigated VHF has been found for Ebola antigen. Titres ranged from 16 to 512 for both Ebola prototype strains tested (Ebola Mayinga from Zaire and Ebola Boniface from Sudan).

Out of 1,083 randomly selected sera, we calculated the difference in dilution titres between the 2 antigens for each of 188 positive sera. In 89 % of the cases, titres were identical, with 69 positive sera having the same titre and 895 sera being negative for both antigens. The average difference in titre among all samples was 0.131 dilution. For the IFA test using a 50 % dilution, it is classical to consider as equivalent titres having one or less difference of dilution. Only 9 sera differed by two dilutions with relatively low titres ($\leqslant 64$) (table III; fig. 2). Such types of reactivity, equivalent titres or slight differences

TABLE III. — **Divergency in titre of sera tested against 2 strains of Ebola: Mayinga from Zaire and Boniface from Sudan.**

N° of divergent dilution		Nb of cases (%)
0	Negative (*)	895 (82.6)
0	Positive (**)	69 (6.4)
1		110 (10.2)
2		9 (0.8)
> 2		0
Total		1.083

(*) Negative sera for both antigens.
(**) Titre > = 16.

in dilution, gave an unusual pattern compared to previous findings in human cases of Ebola disease from endemic areas showing high preferential titres against the indigenous strain (Teepe *et al.*, 1983).

Maroua (379 sera tested) was the only place where we found a significant difference between age group and sex regarding VHF-reactive antibodies. The seroprevalence of age class 0-29 was twice greater (14.7 %) than that for 30-79 year olds (7.8 %) ($X^2 = 24.1$, $df = 1$, $p < 0.01$). Similarly, males were twice as often infected as females ($X^2 = 3.9$, $df = 1$, $p < 0.1$). Previous findings demonstrated a higher prevalence in women than in men in Kenya, Zaire and Uganda (Johnson *et al.*, 1983; B. Larouzé *et al.*, unpublished data). Perhaps there is an unidentified risk factor associated with sex and/or age, involving social behaviour.

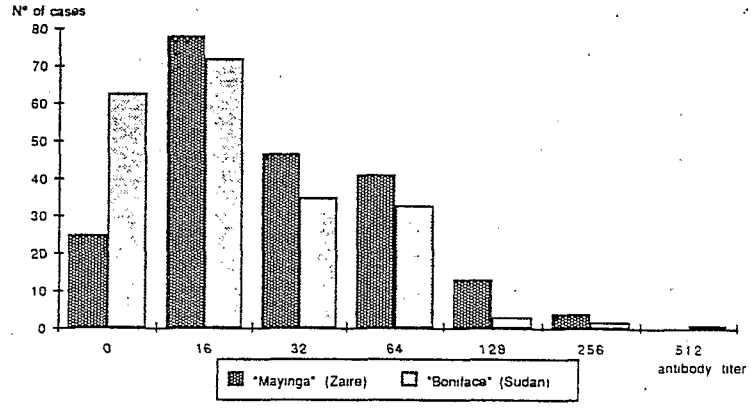

FIG. 2. — *Histogram of titres of 1,083 human sera randomly selected from central Africa and tested by IFA against two Ebola antigens.*

In Mora, Cameroon (395 sera tested distributed in 13 clusters), the highest prevalence of antibody was found in the "piedmont" area (22.5 % positive samples) with a consistent difference between the plain area (6.9 %) and the table land (11.2 %); cluster samples were distributed equally between the two zones ($X^2 = 16.09$, $df = 2$, $p < 0.001$).

Risk factors associated with the environment have not been identified. For this reason, we undertook intensive rodent trapping since crop storage is of major importance in the piedmont area. Of 176 rodents tested, no serological evidence was found in either *Taterillus sp.* or *Mastomys sp.*, the most common peridomestic rodent genus in the area (Gonzalez *et al.*, unpublished data). Up until now, despite numerous surveys, no evidence of a wild or domestic virus reservoir has been found.

The highest prevalence of antibody was found in an area of tropical forest (domains IV and V, fig. 1), but neither epidemics nor clinical cases have been noted, despite the hospital-based survey conducted by OCEAC. Moreover, arid areas like Mora were found to have a consistent antibody prevalence; after 6 years of serological and clinical survey in similar zones of the CAR, we observed significant antibody prevalence without clinical history (Johnson and Gonzalez, unpublished data).

In conclusion, ecological environments distant from those in which epidemics and viruses have been identified, high antibody prevalence in the absence of clinical manifestation, and an unusual pattern of serological response, have led us to hypothesize that a central African Ebola-like virus which is non-pathogenic for humans is in circulation.

Throughout Africa, Marburg antibodies are rare and prevalence is low (< 5 %) (Bergmann and Bourrée, 1982; Gonzalez *et al.*, 1983, Meunier *et al.*, 1987a; Meunier *et al.*, 1987b). The 8 cases from Bioco Island and 11 cases found in Pointe Noire represent 95 % of the total positive sera against Marburg antigen, with a respective prevalence of 2.59 and 3.05 %. We cannot speculate further without identifying risk factors, virus reservoirs or vectors.

Lassa fever.

One positive sample in Bioco and 2 in Mora gave us the lowest antibody prevalence out of all the antigens tested. Rodent trapping in Mora produced some specimens with Lassa antibodies (4 of the 126 *Mastomys sp.* tested). Sera with titres > 32 reacted most with Lassa virus, only slightly with Mobala virus, and did not react with Ippy virus; both arenaviruses are non-pathogenic for humans and were isolated from rodents in CAR (Digoutte, 1970; Gonzalez *et al.*, 1983; Meunier *et al.*, 1985). Positive rodents were found in the piedmont of Mandara mountains close to Nigeria where intensive trading activity between North Cameroon and the Nigerian border exists (50 km from Mora). The low rate of positivity in *Mastomys* compared to that found in endemic areas (> 20 %) led us to suppose that positive human sera could be cases imported from endemic areas such as Nigeria.

As we demonstrated previously (Gonzalez, 1986), Lassa virus appears to be absent from central Africa and its ecological niche is occupied by arenaviruses which are non-pathogenic for humans. Thus, in agreement with the present data observed in North Cameroon and our previous findings (Gonzalez and McCormick, 1987), we propose the following hypothesis on the geographical partitioning of a Lassa complex spread in Africa.

On the western side of the Mandara mountains (north of Cameroon) and the Adamaoua chain (south of Cameroon), endemic transmission of Lassa fever occurs from Nigeria to Sierra Leone (Monath, 1975). On the eastern side of these mountains, several arenaviruses are present and remain enzootic in rodents. Perhaps these mountains represent a natural barrier between Lassa

fever of West Africa and the non-human pathogenic arenaviruses of central and austral Africa.

We previously described the possible segregation and subsequent co-evolution of host and virus isolated by natural barriers creating a Lassa complex at a continental level (Gonzalez and McCormick, 1987). Perhaps positive human cases from Bioco Island should be investigated to demonstrate either their imported status or the presence of another arenavirus that is non-pathogenic for humans.

HFRS.

Consistently high seroprevalence has been found in all places investigated, with the highest rate in Nsork.

In Cameroon, the only area explored (Nkongsamba) showed 3 positive human sera (0.79 %). In Mora (Cameroon), out of 176 rodents tested *(Mastomys* and *Taterillus),* we found one, *Taterillus sp.,* which was positive against the Hantaan virus antigen; it is known that this rodent group represents a candidate reservoir because of its ability to be experimentally infected by hantavirus (J.B. McCormick, pers. comm.).

All 4 districts, Libreville, Port Gentil, Ngounié and Haut Ogooué, in Gabon have significant antibody prevalence. A negative area, Makokou (Ogooué-Ivindo district), may be found to have a specific environnement which excludes risk factors associated with HFRS. The overall rate of 6.15 % of antibody prevalence for the country is fairly comparable to that observed in CAR (7.9 %) where hantavirus appears to be endemic and where the first human case of HFRS in Africa was described (Coulaud *et al.,* 1987; Gonzalez *et al.,* 1988). A study targeted on Ambinda village (Haut Ogooué, Gabon) gave an antibody prevalence of 8 %. Virus activity was deduced by the presence of specific anti-Hantaan-virus IgM, creatinine elevation and renal dysfunction in positive specimens (Dupont *et al.,* 1987).

As early as 1981, we described evidence for Hantaan-related virus in Africa (Gonzalez *et al.,* 1984). Although *Rattus rattus* and *Mastomys* sp. have been identified as a potential virus reservoirs for hantavirus in Africa, the virus strain has not yet been isolated (Saluzzo *et al.,* 1985). Our observations extend the range of the Hantaan-like virus which appears to be present all over Africa, as suggested by other serosurveys from north and west Africa (Gonzalez *et al.,* 1984; Saluzzo *et al.,* 1985; Fleury *et al.,* 1987).

VHF has a specific epidemiology based on both the pathogen and the environment where the virus circulates. Recent manifestations of CCHF in Africa have suggested that we should give more attention to this rare, but often fatal, human disease. The epidemiology of CCHF virus in Africa has been documented only recently with a few human cases observed during the past decade. The distribution of CCHF in Africa appears to overlap that of cer-

tain tick species, especially of the *Hyalomma* genus. Nevertheless, the virus reservoir and maintenance cycle are unknown.

Although RVF virus appears to be less pathogenic for humans than CCHF virus, it is highly infectious and can produce evolutive epidemic and enzootic manifestations. Recent epidemics of RVF in West Africa have inspired serosurveys in areas at risk (Guillaud *et al.*, 1988). The Senegal River basin represents one such site where small ruminants have been shown to be an effective marker of epizootic manifestations prior to the epidemic (Jouan *et al.*, 1988). Vaccines have been developed and are in the experimental phase 4 in West Africa for both human and animal protection.

Filoviruses are known to be highly pathogenic for humans and primates. Strains have been isolated only during a few epidemics and from rare sporadic cases. No strains have been isolated in endemic zones during interepidemic phases. Several domestic and wild animals appear to have Ebola antibody. However, no potential reservoir has yet been identified. Even in central Africa, where human cases have been found, virus has been isolated and high seroprevalence was observed, the maintenance cycles of these filoviruses remain cryptic.

Lassa fever appears to be absent from these regions of Africa and self-limited in other geographical zones where both human-pathogenic and rodent-specific strains are present. Nevertheless, the risk of imported cases exists because of the proximity of endemic areas. Lassa fever appears to be endemic in several countries of West Africa and remains an important public health concern.

Hantavirus strains appear to be present in Africa but HFRS seems to be a mild clinical syndrome, as is observed in occidental Europe. African virus isolates are needed to further investigate the spread of the virus in Africa and to define its clinical involvement.

When virus is present and ecological factors are conducive, VHF can quickly emerge without warning and develop into an epidemic with a high potential of infectivity. To better understand the spread and self-limiting nature of the epidemics, it will be necessary to conduct serosurveys across Africa in identified zones of targeted risk populations.

RÉSUMÉ

PRÉVALENCE EN ANTICORPS CONTRE LES VIRUS DES FIÈVRES HÉMORRAGIQUES DANS DES ÉCHANTILLONS DE POPULATION REPRÉSENTATIFS DE PAYS D'AFRIQUE CENTRALE

De janvier 1985 à juin 1987, des échantillons de population statistiquement représentatifs de zones ciblées dans différents écosystèmes ont été prélevés dans six pays d'Afrique Centrale (Cameroun, Congo, Gabon, Guinée Equatoriale, Tchad et République Centrafricaine): 5070 sérums ont pu être testés par immunofluorescence indirecte contre les antigènes des virus responsables des fièvres hémorragiques les plus

répandues en Afrique. La présence d'anticorps pour chacun de ces virus a été détectée à des taux variables en fonction de l'agent pathogène et des zones explorées: fièvre de Crimée-Congo (0,22 %), fièvre de la Vallée du Rift (0,18 %), maladie d'Ebola (12,40 %), maladie de Marburg (0,39 %), fièvre de Lassa (0,06 %) et *fièvre hémorragique avec syndrome rénal* (6,15 %).

MOTS-CLÉS: Fièvre hémorragique; Prévalence sérologique, Afrique Centrale.

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