EPIDEMIOLOGICAL AND EPIZOOTIOLOGICAL INVESTIGATIONS OF FILOVIRUSES IN THE CENTRAL AFRICAN REPUBLIC

FINAL REPORT

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A member of the filovirus group is active in the CAR, as demonstrated by significant seropositivity rates in human populations as well as in different animal species (rodents, dogs, cattle, chicken). Infection by this virus seems not usually associated with severe illness or hemorrhagic signs.
Several cases of hemorrhagic fevers have been diagnosed in the CAR, due to Yellow fever and Rift Valley fever viruses. Congo-CHF virus and 2 members of the Arenavirus group are also present in the CAR.
Further studies are needed : 1) isolate and characterize the member of filovirus group, 2) establish the biological significance of filovirus antibodies, 3) define the epidemiology of filoviruses in CAR, 4) assess clinical and epidemiological biohazard of hemorrhagic fever viruses in Central Africa.

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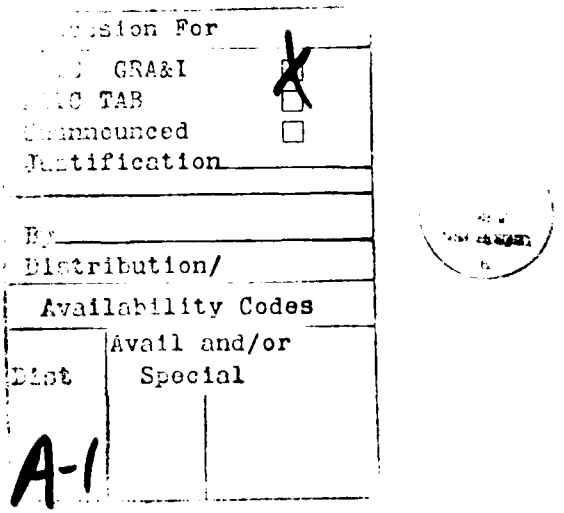
S U M M A R Y

The collaborative program of sero-epidemiological investigations on Central African Hemorrhagic Fever Viruses (specially Filoviruses), has been focused in 1987 and 1988 on two cooperative villages, Gordil and Ouandja, of the Vakaga district (Northen part of the country), which have been studied since 1985.
There are serological evidences of the active circulation of an antigenically related member of the Filovirus group. The infection can occur early in the life and inter-human contamination is not to be excluded.
As very few cases of severe illness seems to be associated with infection by this virus in the CAR, and as the antigenic specificity of the serological test used is not known, one cannot assert if the virus responsible for the high rate seropositivity is Ebola, Marburg or an antigenically related strain.
For similar reasons, despite the obtention of numerous serological and virological data, the role of animals (rodents, cattle, chicken, goats, sheeps, dogs) in the natural cycle of filoviruses cannot be still clearly established.
Further studies are needed : to isolate this virus, to determine whether the antibodies detected are specific of Ebola or Marburg virus, or can neutralize these viruses, and to study the Filovirus epidemiology in the CAR by establishing a meticulous survey of seropositive and seronegative families.

During the same period Institute Pasteur's own research was active in others places than the Vakaga's district. Data on Hemorrhagic Fever viruses and arboviruses activities from other CAR's ecosystems have been obtained. The spreading of Filovirus in the surrounding countries i.e. Chad, Congo, Zaïre, Equatorial Guinea, Gabon (and also Madagascar, far from CAR) has been assessed, at least, partially.
Isolations of viral strains have been made available resulting from the intense surveillance system organized in several places of the CAR since 1981.



FOREWORD

For the protection of human subjects, the investigators have adhered to policies of applicable Federal Law 45CFR46.

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BODY OF THE REPORT

A. STATEMENT OF THE PROBLEM

In the past, the filoviruses, a new group of pathogenic agents, were not adequately or thoroughly studied in Africa. The current Institute Pasteur/USAMRIID filovirus field program was established to systematically study these agents and accurately assess and reduce their natural threat by defining their epidemiology, ecology, pathogenicity, and by developing procedures to control disease.

The current field program, as outlined in previous reports (1,2), was divided into three phases. Phase one consisted of cross-sectional serosurveys conducted in different ecological or phytogeographic areas to locate areas of virus activity as measured by the prevalence of filovirus reactive antibody. Phase two consisted of prospective studies conducted in well defined populations to document active virus transmission by identifying seroconversions. Phase three consisted of in depth studies to identify the occurence of clinical diseases associated with antibody prevalence and define the local ecology of filovirus activity. Phase one was completed and phase two started in 1986. The results of the phase one studies on the epidemiology of filoviruses in CAR (Central African Republic) suggested that the research efforts should be formed on the Vakaga district in the northern part of the country : 1. The filovirus activity as measured by antibody prevalence was highest in this region; 2. Virus activity was confined to select villages; 3. The antibody prevalence was highest among the female population; 4. Both Ebola virus and Marburg virus were active in the Vakaga district; and 5. The numerous double seropositives (Ebola virus and Marburg virus reactive antibody positives) suggested shared risk factors for the filoviruses.

Unfortunately, our follow-up study revealed that conducting long term prospective studies in the Vakaga would be difficult. Many villages, Amardjedi and Sikikede for example, were not willing to participate in additional studies. The reluctance of Amardjedi and Sikikede residents to participate in long term studies was due to their support of illegal poaching and their fear that outsiders would notify the authorities of their activities.

In light of the reluctance of many villages to cooperate and the logistical problems encountered in traveling deep into the Vakaga district, two cooperative villages, Gordil and Ouandja, were selected for detailed studies in 1987 and 1988. The results of cross sectional studies revealed that the antibody prevalence in villages that neighbored Gordil and Ouandja did not significantly differ from the antibody prevalence in Gordil and Ouandja, indicating that the results generated from studies in these two villages would reflect the epidemiology and ecology of the region.

Unfortunately we encountered also in 1988 the reluctance of inhabitants of Gordil, as they told us they had been promised by previous mission staff to be provided with wells and other goods. Of course it was impossible to dig wells considering our capacities.

Taking account of the results of the 1987 serosurvey (3), we focused in 1988 our studies on select populations : children less than 6 years old, families with seroconversion cases or with numerous seropositives, dogs, rodents caught in fields owned by seropositive and seronegative families.

This Filovirus Research program inVakaga is only a part of the Institute Pasteur research on hemorrhagic fever viruses. Numerous serological data have been obtained from other places in and out the CAR. Moreover a special surveillance program, including physicians, virologists and entomologists, has been established to detect and identify the circulation of hemorrhagic fever virus and pathogenic arboviruses.

B. METHODS

1. FILOVIRUS RESARCH PROGRAM IN VAKAGA :

1.1. Sampling procedures:

In brief, the following sampling procedure was implemented. In each village, volunteers were selected, questionnaires consisting of general background questions were completed for each individual, blood samples were drawn, and each participant was given an identification number, medical examination, and treated for illnesses. Children less than 6 years old included in the 1988 survey were vaccinated against Yellow Fever. In each village a preliminary census has been conducted, maps drawn and heads of all households identified.

Blood samples were also taken on different animal species, especially animals living in contact with man : rodents, dogs, chickens, cattle.

1.2. Serological procedures:

Blood samples were drawn and allowed to clot and the resulting serum specimen was divided into two aliquots. One aliquot was screened at the Institute Pasteur and one aliquot was tested at USAMRIID. The serum samples were thawed and separated into three aliquots. Two aliquots were stored frozen at -20°C, while the remaining aliquot was diluted and screened double blind by IFA for hemorrhagic fever virus reacting antibodies. The samples were screened at a 1:16 dilution on CRELM slides and the seropositives were screened and titrated on hemorrhagic fever virus infected and uninfected Vero cell monospecific spot slides.

Specimens were considered positive if they reacted with virus infected and not virus uninfected Vero cell monospecific spot slides. Specimens were considered positive if they reacted with virus infected and not virus uninfected Vero cells. The endpoints for the serological titrations were recorded as the reciprocal of the last dilution which produced a positive react with infected cells. The serological results cited in reports are those obtained at USAMRIID Laboratory, but one also will found the Institute Pasteur's own results obtained in 1985,1986, 1988.

In 1988, not only the same sera found to be positive by USAMRIID laboratory were positive too in our hands, but also some others which poses the problem of the specificity of the only IFA test for assessing Filovirus activity.

2. SURVEILLANCES PROGRAM :

In parallel with the Vakaga's district surveillance, a pertinent program of research of possible Filovirus activity has been conducted in other places, in and outside the CAR, including research for other viruses known to be responsible for hemorrhages (RVF, YF, Lassa, etc..) and Arboviruses as well.

2.1. Collection of samples :

The collection of samples was as follows:

2.1.1. **Human samples**

2.1.1.1. Survey areas

Human sera were collected by physicians participating to the cooperative program for the surveillance and diagnosis of diseases due to Hemorrhagic Fever Viruses. Cooperation had been previously established with physicians practising in Bangui and out of Bangui: e.g. physicians from the Centre National Hospitalo-Universitaire, the Clinique Chouaib, the Medical Center of the French Embassy, the Army Medical Center, and private practitioners as well. Out of Bangui a cooperation is established (since 1980) with the hospital in Bouar, a town of 100,000 inhabitants situated 450 km north-west of Bangui in a moist sub-Sudanese savanna area, and with the Tandala Mission Hospital, located in the transition zone of tropical rain forest to savanna in north Zaire, and where a fatal case of hemorrhagic fever due to Ebola virus occured in 1977.

2.1.1.2. Methods

Patients presenting with symptoms compatible with an acute viral infection, i.e. febrile syndrom without active malaria, and accompanied with at least one other clinical symptom suggesting a possible virus involvement such as algia, hemorrhage, hepatitis, renal failure, encephalitis, shok syndrom, were immediatly bled by venipuncture for virus isolation attempt and serology. The patients were asked to be rebled 3 weeks later for serology. Blood samples obtained in Bangui were transported immediatly at the Institut Pasteur for separation of serum. The sera were forthwith tested for virus or stored in at -70°C until treatment. Blood samples collected in the centers out of Bangui were allowed to clot in the local laboratory, then the sera stored in liquid nitrogen until shipment to the Institut Pasteur in Bangui.

2.1.2. **Arthropodes collections**

The surveillance was first established for the control of the circulation of yellow fever virus. It allowed in fact the isolation of numerous arboviruses, including, besides yellow fever virus, several pathogenic viruses such as West-Nile, Chikungunya, or more recently Semliki-Forest viruses, and two viruses responsible for haemorrhagic fever syndroms, Congo-Crimean haemorrhagic fever and Rift Valley fever viruses.

2.1.2.1. Places of surveillance

The surveillance was principally centred on two locations: Bangui and Bozo.

The ecological surveillance in Bangui was performed in 4 different places: the international airport area, the woody hill area, and 2 dwelling quarters.

Bozo is a village of 200 inhabitants situated 100 km north of Bangui in the sub-Sudanese savanna zone, with a gallery forest 5 km from the village.

2.1.2.2. Methods

Human bait mosquito collections are carried out 3 times a month in each place of Bangui chosen for the survey, and during 4-day long field missions each month in Bozo. Mosquitoes are caught individually in a glass tube, classified by species, anaesthetized with chloroform, grouped in pools of 10 to 90 mosquitoes, and stored in liquid nitrogen until they are tested for virus isolation.

2.1.3. Vertebrates

According to the results of vectors surveillance or clinical investigations, potential vertebrate hosts for virus were investigated. Different animal species have been tested, including rodents, caught in Sherman traps, monkeys, and domestic animals (dogs, cattle, sheeps, ...).

2.2. Virus isolation :

Pools of mosquitoes or animal organs at 1:10 dilution in Hanks balanced salt solution pH 7.2 supplemented with 10% fetal calf serum, 100 U penicilline and 50 µg streptomycin per ml, are triturated in cold mortars. Triturated pools are clarified by centrifugation at 700 x g for 10 mn at +4°C. Eight 2-day old Swiss mice are inoculated both intracerebrally and intraperitoneally with 0.015 ml of supernatant. Inoculated mice are examined daily for 18 days. Mice presenting with suggestive signs such as tremors, paralysis, lying on side, unusual activity, wasting, are considering to be suffering from virus infection and harvested. Brain suspensions of infected mice are passaged until the virus is adapted to mice. The virus strain consists of the clarified suspension of infected mice brain triturated at 1:10 dilution in Hanks + 10% FCS + antibiotics.

Human sera are diluted at 1:10 in Hanks + FCS + antibiotics, inoculated in new-born mice using the same procedure as to mosquitoes, and on Vero E_6 cells cultures. Volumes of 0,5 ml diluted sera are inoculated on monolayers of Vero E_6 cells grown in 5 cm^2 culture tubes with Eagle Minimum Essential medium + FCS + antibiotics. After adsorption 1 hour at 37°C cells are fed with maintenance medium (EMEM + FCS + antibiotics). Inoculated cultures are examined daily for cytopathic effect.

2.3. Virus identification

A standard indirect immunofluorescence assay is used for preliminary virus identification on Vero cells showing a cytopathogenic effect after inoculation with diluted human serum or with clarified infected mice brain suspension. Cells without CPE 7 days after inoculation of human serum are also systematically tested.

Culture tubes are decanted, cells are washed with PBS pH 7.2, then suspended in 1 ml PBS. Twenty microliters of cells suspension are placed on ten-spot microscope slides. Cells are air-dried, fixed in acetone at -20°C and stored at -70°C until tested.

A panel of 20 mouse immune ascitic fluids prepared against the most common arboviruses in CAR, i.e. chikungunya, Igbo Ora, Middelburg, o'nyong nyong, Semliki Forest, Sindbis, Bouboui, Kedougou, Usutu, Wesselsbron, West-Nile, yellow fever, Zika, Congo-CHF, Rift Valley fever, Bwamba, Dugbe, Ilesha, Tataguine, and Mengo viruses, is used as primary antibodies. Monoclonal antibodies against Congo-CHF, RVF, Ebola, Marburg, Lassa, Hantaan viruses (USAMRIID) are also used. All sera are used at 1:16 dilution in PBS. The second antibody is a commercial sheep anti-mouse IgG,A,M antibody conjugated to fluoresceine isothiocyanate used at 1:100 dilution in PBS + Evans blue.

Arbovirus identification is confirmed by the WHO Centre for Arbovirus Reference and Research, Dakar, Senegal. Confirmation of Ebola, Marburg, Lassa or Hantaan virus identification will be made at USAMRIID, Fort-Detrick.

2.4. Serology (Complement to paragraph 2)

Serologic tests are performed by a similar IIF test as that used for virus identification, using slides of Vero cells infected by different virus strains. Slides of "conventional" arboviruses are prepared at Institut Pasteur in Bangui, while polyspecific and monospecific slides of Hemorrhagic Fever viruses (CCHF, RVF, EBO, MBG, LAS, KHF) are furnished by USAMRIID.

Human sera are screened at 1:16 dilution and positive sera are titrated by 2-fold dilutions against the reacting infected cells. The revealing antibody is a commercial goat anti-human IgG,A,M antibody conjugated to FITC, used at 1:100 dilution.

Care is taken for interpretation of serology by IF methods, as results appear to be equivocal, especially concerning filovirus.

C. RESULTS

1. FILOVIRUS ACTIVITY IN THE VAKAGA DISTRICT

1. Filovirus antibody prevalence :

The prevalence seems lower in 1988 than the one observed during the period 1985-1987 (Tables I, II). In Ouandja this antibody decreasing seems to be related to an unusual weak prevalence in females, especially less than 30 years old (Table III), whereas the 1988 prevalence in males is not significantly different from the 1985-1987 prevalence.

Despite this new feature the overall prevalence in Gordil and Ouandja for the period 1985-1988 remains high and similar (22.8% in Gordil, 24.9% in Ouandja). None of the human sera collected in 1988 reacted with Marburg antigen.

An encampment of Mbororo people (nomads travelling with their cattle herd) was also investigated, the overall Filovirus antibody prevalence was 4.5% (1 positive/22 tested).

2. Seroconversions, seroreversions :

Unlike the previous serosurveys (3), no seroconversion has been observed in 1988. On the contrary several seroreversions occured between 1987 and 1988 (Table IV). Especially, some high titered seropositive persons in 1987 were found seronegative in 1988, and 3 persons who seroconverted between 1986 and 1987, then seroreversed between 1987 and 1988.

3. Seroprevalence in select animal populations :

Whereas antibody prevalence in dogs was high in 1987 (3), no dog serum has been found positive in 1988 (Table V). On the other hand the prevalence in rodents is comparable, indicating a low infection level in these mammals caught near houses or in croplands as well.

2. MISCELLANEOUS: ARBOVIRUS and FILOVIRUS OUT OF VAKAGA.

1. Viral isolations :

The table VI gives inventory of viruses isolated during the period 1986-1988.

One can note the presence of virus belonging to several families of arboviruses (4, 5, 6, 7, 8, 9). Mainly Alphavirus, Flavivirus, Nairovirus, Phlebo virus (including 4 highly pathogenic strains of RFV virus), and Arenavirus.

Organs were collected from trapped rodents for filovirus isolation attempts. These experiments were poorly productive since from 494 trapped in the Vakaga District through the 1987-1988 period, 452 were inoculated in both Vero E6 and suckling mice leading to isolation of 2 strains : Ippy (Praomys) and Mengo (Tatera).

Another strain of Ippy virus was obtained from a Praomys trapped in OUHAM (near Bouar) in an ecosystem resembling to Vakaga.

While Human and animal are frequently found positive for Hemorrhagic antibodies Filovirus and Flavivirus or Phlebo virus as well, few significant clinical hemorrhagic fever cases were diagnosed in the CAR. A strong permanent surveillance of 4 Areas :

Vakaga (4 annual trips), Bangui, Bouar and Tandala (located at 150 Km from Bangui in Zaïre), and occasional surveillance of other places as mentionned in table VII, allowed us to diagnose 5 human cases (3 due to RVF Virus, 2 due to Yellow fever virus). One must mention also isolation of West Nile from 4 patients with fatal Hemorrhagic Hepatitis in year 1983-84, before the current study (5, 6, 7, 10).

2. Serological surveys:

Tables VII to XII give a summary of human serological surveys against hemorrhagic fever viruses in CAR and out CAR performed at the Institute Pasteur.

Table I : Filovirus antibody prevalence in 1985, 1986, 1987 and 1988, previously unsurveyed Gordil populations.

Survey Year	LAB Identif*	General Total	General N° Pos	General % Pos	Male Total	Male N° Pos	Male % Pos	Female Total	Female N° Pos	Female % Pos
85	USAMRIID-IPB	147	30	20.4	92	19	20.6	55	11	20.0
86	USAMRIID-IPB	62	10	16.1	27	3	11.1	35	7	20.0
87	USAMRRID	89	34	38.2	33	12	36.4	55	20	36.6
88	USAMRIID	30	0	0.0	17	0	0.0	13	0	0.0
88	I.P. BANGUI	30	3	10.0	17	1	6.0	13	2	15.0

Only those individuals not surveyed in previous years are shown in this table.

*Workers for I.P. Bangui : 1985 Alain J. GEORGES and Jean Paul GONZALEZ, 1986 J.P. GONZALEZ and Didier MEUNIER, 1987 no aliquot available for IPB, hence not tested in Bangui according to joined agreement IPB-WRAIR, 1988: Christian MATHIOT, Alain J.GEORGES.

(a) Same samples than previous data shown by USAMRIID 1988, Not considered for establishing percentages (discrepency).

Table II : Filovirus reactive antibody prevalence in 1985, 1986, 1987 and 1988 previously unsurveyed Ouandja populations.

Survey Year	LAB Identif*	General Total	General N° Pos	General % Pos	Male Total	Male N° Pos	Male % Pos	Female Total	Female N° Pos	Female % Pos
85	USAMRIID-IPB	135	40	29.6	64	12	18.8	71	28	39.4
86	USAMRIID-IPB	105	14	13.3	47	4	8.5	58	11	18.9
87	USAMRIID	219	73	33.3	103	24	23.5	116	49	40.5
88	USAMRIID	79	7	8.8	38	5	13.2	38	2	5.3
88	I.P.BANGUI(a)	76	13	16.0	38	7	18.0	38	6	16.0

Only those individuals not surveyed in previous years are shown in this table.

*Workers for I.P. Bangui : 1985 Alain J. GEORGES and Jean Paul GONZALEZ, 1986 J.P. GONZALEZ and Didier MEUNIER, 1987 no aliquot available for IPB, hence not tested in Bangui according to joined agreement IPB-WRAIR, 1988: Christian MA IOT, Alain J.GEORGES.

(a) Same samples than previous data shown by USAMRIID 1988, Not considered for establishing percentages (discrepency).

Table III : Age and sex distribution of filovirus antibodies in previously unsurveyed populations (1988). (C.MATHIOT, P.M.V.MARTIN, M.C.GEORGES-COURBOT).

Age	Females			Males			General		
	Total	N° Pos	% Pos	Total	N° Pos	% Pos	Total	N° Pos	% Pos
01-06	20	0	0.0	24	3	12.5	44	3	6.8
06-10	4	0	0.0	7	1	14.3	11	1	9.1
11-20	5	0	0.0	2	0	0.0	7	0	0.0
21-30	2	0	0.0	0	0	0.0	2	0	0.0
≥ 30	7	2	28.6	5	1	20.0	12	3	25.0

Table IV : Filovirus seroreversions in Ouandja and Gordil between 1986 and 1988.(C.C.MATHIOT, P.M.V.MARTIN, M.C.GEORGES-COURBOT)

House	sex / age	1986			1987			1988		
		Es	Ez	Mbg	Es	Ez	Mbg	Es	Ez	Mbg
Ouandja:										
12	M/7	0*	0	0	32	256	0	0	0	0
13	M/11	0	0	0	128	128	0	0	0	0
14	M/65	16	16	0	64	128	0	0	0	0
24	M/16	NT	NT	NT	32	128	0	0	0	0
39	M/10	NT	NT	NT	1024	1024	0	0	0	0
	M/8	NT	NT	NT	128	256	0	0	0	0
46	F/35	NT	NT	NT	128	512	0	0	0	0
48	F/41	64	128	0	256	512	0	0	0	0
54	F/41	16	32	0	128	128	0	0	0	0
72	M/15	0	0	0	128	512	0	0	0	0
	F/9	NT	NT	NT	64	0	0	0	0	0
84	F/33	32	64	0	256	256	0	0	0	0
	M/12	NT	NT	NT	32	128	0	0	0	0
	M/6	NT	NT	NT	128	64	0	0	0	0
Gordil:										
B16	F/9	NT	NT	NT	128	32	0	0	0	0

* Titres against Ebola Sudan (Es), Ebola Zaïre (Ez), Marburg (Mbg)
NT not tested

Table V : Prevalence of Filovirus reactive antibody prevalence in animal serosurveys 1987 and 1988

SURVEY SPECIES	YEARS SURVEY	TOTAL N°	FILOVIRUSES POS N°	FILOVIRUSES POS %	CCHFV POS N°	CCHFV POS %	RVFV POS N°	RVFV POS %	LASSA POS N°	LASSA POS %
Cattle	87	20	2	10	4	20	0	0	0	0
	88	26	4	15.4	3	11.5	0	0	0	0
	87	131	5	3.8	0	0	0	0	4	3.1
Chickens	88	50	1	2	0	0	0	0	0	0
Dogs (a)	87	29	10	34.5	0	0	0	0	0	0
	88	23	0	0.0	0	0	0	0	0	0
Donkeys	87	13	3	23.1	0	0	0	0	0	0
Goats	87	75	0	0	0	0	2	2.7	0	0
Sheeps	87	17	0	0	1	5.9	1	5.9	0	0
Rodents	87	379	17	4.5	0	0.0	0	0.0	2	0.5
	88	115	2	1.7	0	0.0	0	0.0	2	1.7
Mastomys	87	265	12	4.5	0	0.0	0	0.0	2	0.8
	88	67	1	1.4	0	0.0	0	0.0	0	0.0
Arvicanthis	87	98	9	9.2	0	0.0	0	0.0	0	0.0
	88	36	3	8.3	0	0.0	0	0.0	0	0.0
Shrew	87	5	0	0.0	0	0.0	0	0.0	0	0.0
	88	1	0	0.0	0	0.0	0	0.0	0	0.0
Taterillus	87	4	0	0.0	0	0.0	0	0.0	0	0.0
	88	6	0	0.0	0	0.0	0	0.0	0	0.0
Praomys	87	3	0	0.0	0	0.0	0	0.0	0	0.0
	88	4	0	0.0	0	0.0	0	0.0	0	0.0
Other (b)	87	4	0	0.0	0	0.0	0	0.0	0	0.0
	88	1	1	0.0	0	0.0	0	0.0	0	0.0

a) Village prevalence (1987): 2/7 (28.5%) Gordil, 8/22(36.4%)Ouandjia. (1988): 0/5 Gordil (0%) and 0/18 Ouandjia (0%).

b)(1987) Myomys, Gerbil, Lemniscomys, Grapiosus; (1988) Aetomys.

TABLE VI: Virus isolations from 1986 to 1988

GROUPE et GENUS	VIRUS	ORIGIN	PLACE OF COLL:*
ALPHAVIRUS			
Middelburg	Ar tB 5290	Amblyomma variegatum	BANGUI (OP)
Semliki Forest	22 Strains	Human	BANGUI (OP)
Semliki Forest	25 Strains	Mosquitoes	BANGUI AND BOZO
FLAVIVIRUS			
Yellow Fever	R 1504	Human Serum	KAPOU (OP)
	HB1782	" "	BERBERATI (HS)
Zika	4 Strains	A.Africanus	BOZO (OP)
Bouboui	12 Strains	A.Africanus	BOZO (OP)
NAIROVIRUS			
Dugbe	Ar tB 2549	Eggs of A.variegatum	BANGUI (OP)
	Ar tB 5361	Amblyomma variegatum	BANGUI (OP)
PHLEBOVIRUS			
Rift Valley Fev.	R 1752	Human Serum	BANGUI (OP)
	R 1662	Human serum	BANGUI (OP)
	HVB 375	Human serum	BANGUI (OP)
	HB 4828	Human serum**	BANGUI (OP)
Gabek Forest	An RB 3748C	Taterillus sp	MAITOUKOUL(OHM) (Ouham)
Bwamba	HB 83 P48	Sérum Humain	BANGUI (OP)
ARENAVIRUS			
Ippy	An RB 4194	Praomys sp	BALEGOU (OU)
Ippy	An RB 4623	Praomys sp	GORDIL (VAK)
OTHERS			
Mengo	AnRB 4769	Tatera	GORDIL (VAK)

*
OP : OMBELLA MPOKO
HS : HAUTE SANGHA
OU : OUAKA
OHM : OUHAM

** Non hemorrhagic fever

TABLE VII: SEROLOGICAL SURVEYS OF FILOVIRIDAE IN HUMAN IN CENTRAL AFRICA THROUGH A 4 YEARS PERIOD, (VAKAGA AREA EXCLUDED).

PLACE	COUNTRY	YEAR	TOTAL TESTED	EBOLA ZAïRE		EBOLA SUDAN		MARBURG	
				Nb	%	Nb	%	Nb	%
BALEMBE (NM)	CAR	85	31	2	6.5	1	3.2	1	3.2
BOUAR (NM)	CAR	85	112	9	8	7	6.3	1	0.9
DIKA (NM)	CAR	85	75	9	12	8	10.7	5	6.7
DONGO-BOD. (NM)	CAR	85	59	2	3.4	1	1.7	1	1.7
NDONGUE (NM)	CAR	85	10	0	0	0	0	0	0
ZEMIO (HM)	CAR	85	140	61	43.6	0	0	0	0
BAMBOUTI (HM)	CAR	85	82	50	61	0	0	0	0
BAGANDOU (L)	CAR	85	130	7	5.4	45	34.6	1	0.8
ABINDA	GABOON	85	213	19	9	2	1	0	0
MOROUBA (BK)	CAR	86	95	3	3	5	5.3	0	0
BRIA (HK)	CAR	86	285	57	20	52	18.2	1	0.35
CHAMBOULI	CHAD	86	81	0	0	0	0	0	0
NGOUNIE	GABON	86	249	57	22.9	57	22.9	0	0
MALANGA	CHAD	86	72	16	22	15	21	0	0

(NM) NANA MAMBERE
(HM) HAUT MBOMOU
(L) LOBAYE
(O) OUAKA
(BK) BASSE KOTO
(HK) HAUTE KOTO

TABLE VIII: SEROLOGICAL SURVEYS IN CAR OF FILOVIRUS ACTIVITY IN HUMANS IN OMBELLA MPOKO, INCLUDING BANGUI, THROUGH A 4 YEARS PERIOD (85-88).

	TOTAL	EBOLA ZAïRE		EBOLA SUDAN		MARBURG	
1985	112	11	10	14	12.5	0	0
1986	239	9	3.8	10	4.2	1	0.4
1987	243	5	2.06	11	4.5	0	0
1988	214	11	5.1	8	3.7	2	1
TOTAL :	808	36	4.46	43	5.3	3	0.37

TABLES IX : REPARTITION OF TITERS OF POSITIVE SERA FOR HEMORRHAGIC FEVER VIRUS IN OMBELLA MPOKO (INCLUDING BANGUI) CAR, THROUGH A 4 YEARS PERIOD.

IXA : YEAR 1985 ; TOTAL TESTED : 112

TITER / VIRUS	1/16	1/32	1/64	1/128	1.256	1/512	Nb CASE	%
Congo	1	1	1	0	0	0	3	2.7
Rift	0	2	0	0	0	0	2	1.8
Ebola Zaïre	6	2	1	1	1	0	11	10
Ebola Soudan	7	3	2	1	1	0	14	12.5
Lassa	0	2	0	0	0	0	2	1.8
Marburg	0	0	0	0	0	0	0	0

IXB : YEAR 1986 ; TOTAL TESTED : 239

TITER / VIRUS	1/16	1/32	1/64	1/128	1.256	1/512	TOTAL POSITIVE	%
Congo	0	0	0	0	0	0	0	0
Rift	0	0	0	0	0	0	0	0
Ebola Zaïre	5	2	1	1	0	0	9	3.8
Ebola Soudan	5	3	1	1	0	0	10	4.2
Lassa	0	0	0	0	0	0	0	0
Marburg	1	0	0	0	0	0	1	0.4

IXC : YEAR 1987 ; TOTAL TESTED : 243

TITER / VIRUS	1/16	1/32	1/64	1/128	1.256	1/512	TOTAL	%
Congo	0	2	0	0	0	0	2	0.8
Rift	0	1	0	0	0	0	1	4.4
Ebola Zaïre	2	2	1	0	0	0	5	2.1
Ebola Soudan	6	4	1	0	0	0	11	4.5
Lassa	0	0	0	0	0	0	0	0
Marburg	0	0	0	0	0	0	0	0

IXD : YEAR 1988 ; TOTAL TESTED : 214

TITER / VIRUS\	1/16	1/32	1/64	1/128	1.256	1/512	1/1024	Nb CASES	%
Congo	0	1	2	1	1	0	1	6	2
Rift	1	0	0	0	0	0	0	1	0.4
Ebola Zaïre	4	3	1	3	0	0	0	11	5.1
Ebola Soudan	2	3	2	0	1	0	0	8	3.7
Lassa	0	1	0	0	0	0	0	1	0.4
Marburg	0	1	1	0	0	0	0	2	1

TABLE X : HUMAN HAEMORRHAGIC FEVER SEROLOGY IN DIFFERENT AREAS OF MADAGASCAR (II).

	ANTIGENS[a]					
Area	CON	RVF	EBOZ	EBOS	MBG	LAS
Antananarivo	0/45[b]	0/45	6/45	0/45	045	0/45
Mandoto	2/149	1/149	7/149	0/149	0/149	0/149
Antsirabe	0/56	0/56	3/56	0/56	0/56	0/56
Tsiroanomandidy	0/105	0/105	0/105	0/105	0/105	0/105
Anmpijoroa	0/26	O/26	1/26	0/26	0/26	0/26
Totals	2/381	1/381	17/381	0/381	0/381	0/381
Percentage	0.5	0.3	4.5	0	0	0

[a]CON = Congo-Crimean haemorrhagic fever, RVF=Rft Valley fever, EBO Z= Ebola (Zaïre), EBO S=Ebola (Sudan), MBG=Marburg, LAS=Lassa fever.
[b]Number positive/Number tested.

TABLE XI : ANTIBODY PREVALENCE AGAINST EBOLA VIRUS IN HUMAN POPULATIONS OF CHAD

Origin	Total tested	Percentage of positive	
		EBO Mayinga	EBO Boniface
Ndjamena	288	3.5	2.1
Sahr	81	30.9	0.0
Moundou	98	34.7	0.0
Goz Beida	45	48.9	0.0
Abeche	48	25.0	2.0
Total	560		

TABLE XII : PREVALENCE RATE OF FLUORESCENT ANTIBODY AGAINST HAEMORRHAGIC FEVER VIRUSES IN CENTRAL AFRICA.

Locality	Phyto-geogra-phycal zones	Sample size	Percentage cf positivity on antigens tested					
			CCHF	RVF	EBO	MBG	LAS	HANT
Ndjamena$	I	334	0.0	0.3	3.5	0.3	0.0	4.2
Mora	I	395	0.5	0.25	10.8	0.0	0.5	NT
Maroua	II	379	0.0	0.0	10.3	0.0	0.0	NT
Nkongsamba	IV	378	0.0	1.1	1.9	0.0	0.0	0.8
Bangui	IV	327	0.0	0.0	32.9	0.0	0.0	NT
Brazzaville	IV	368	0.0	0.0	6.2	0.0	0.0	NT
Bioco Island	V	308	1.3	0.0	16.1	2.6	0.3	NT
Pointe Noire	V	360	1.1	0.0	7.7	3.1	0.0	NT
Port Gentil	V	376	0.0	0.5	7.4	0.0	0.0	8.5*
Nsork	V	380	0.3	0.3	16.0	0.0	0.0	14.2
Libreville	V	349	0.0	0.0	12.0	0.0	0.0	8.4
Makokou	V	360	0.0	0.0	21.7	0.0	0.0	0.0
Mouila (Ngounié)	V	372	0.0	0.0	14.7	0.0	0.0	9.1
Haut Ogoué	V	384	0.0	0.0	14.7	0.0	0.0	0.7**
Total	-	5070	0.2	0.2	12.6	0.4	0.06	6.8***

* 301 sera tested
** 419 sera tested
*** age group
**** 2533 sera tested
$ Different from Ndjamena populations data presented in table XII, i.e. randommized populations 15-60 years of age.

D. DISCUSSION

Collectively, our serological data indicate that the filoviruses (Ebola virus, Marburg virus, or a serologically related member of the group) are active in the Central African Republic. Significant antibody prevalences are consistently observed in select populations and exposure seems to occur early in life. The prevalence of antibodies in children less than 6 years old, and the fact that several families have numerous positive members whereas some families are completely seronegative are consistent with possible occurence of inter-human contamination.

The circulation of a member of the filovirus group seems not to be associated with notable hemorrhagic disease.

In year 1988, we could interrogate 109 persons in North Vakaga district, and among them, 3 persons who seroconverted between 1986 and 1987, they all denied having suffered of a severe illness during this period, and in particular never experimented hemorrhage signs (the 3 persons were found seronegative in 1988).

In the Ombella Mpoko district (surrounding Bangui), during the same period we examined and bled 214 febrile people : none claimed to have any hemorrhagic signs.

We must admit that infection by a member of filovirus group can be associated with no illness or with a mild febrile syndrom, indifferenciable from malaria or "dengue-like" syndrom. Except for one fatal case observed in Gordil in 1985, no severe febrile illness compatible with infection by a virus responsible for hemorrhagic disease has been reported by the inhabitants of Ouandjia and Gordil.

These observations don't exclude the possibility of a Filovirus activity and especially Ebola or Marburg virus since neither the severity nor the clinical spectrum of natural occuring infections have been definitively described. A continuous clinical, epidemiological, virological surveillance remains necessary.

Animal hosts for the filoviruses have been systematically sought. On the basis of species in which antibodies have been detected, cattle, chicken, dogs, donkeys, rodents can be infected in natura by a filovirus. However their possible role in the virus transmission to man is not easy to assess on the sole basis of serological results obtained by IFA test. As for human no filovirus isolation has been obtained from animals.

As regards to cattle, the low prevalence in a group of M'bororo people, living in contact with their livestock herd and who were present in Vakaga for at least 3 years, is not in favour of an active role in the virus transmission. This observation must be confirmed.

Rodents were especially studied because their important role in the transmission of numerous viruses. A low prevalence was observed in 1987 among rodents caught in the village and living in contact with man. In 1988 rodents were caught in croplands owning to families with numerous seropositive and in croplands owning to families with no seropositive: no difference has been found as regards to the seropositivity rate in both rodents populations, suggesting that rodents may don't play an essential role in the transmission of filovirus to man. As our capacities did not permit to study several important factors such as seasonal vegetation, annual rainfall, predator frequency, rodents densities ..., this observation must also be confirmed by longer field missions and assistance of a mammalogist.

Results are not so clear concerning dogs : a high prevalence rate obtained in 1987 (36.4% in Ouandja) led us to suggest the possibility of a virus cycle including dogs. Since this high positivity rate was not confirmed in 1988, further studies are needed to evaluate the role of dogs in the virus cycle.

E. RECOMMENDATIONS

The goal of the collaboration between the Institut Pasteur in Bangui and USAMRIID was to define the epidemiology, ecology and pathogenicity of the filoviruses.

The main following results have been obtained :

- an agent antigenically related to the Filovirus group is active in the Vakaga area in Central African Republic (as well as in other regions), as demonstrated by high seroprevalence rates in human population;

- as very few hemorrhagic diseases are noticed to the Institute Pasteur despite a continuous surveillance program, an infection by this agent doesn't seem usually the cause of notable hemorrhagic disease. On the basis of numerous interviews of seropositives, an infection by this virus seems usually not associated with a severe illness;

- despite numerous isolation attempts, especially from sera samples collected on patients presenting at least an acute febrile syndrome compatible with a virus infection, no filovirus strain has been isolated. One cannot say if the agent responsible for positive serologies is Ebola, Marburg or an unknown antigenically related strain;

- infection occurs early in the life, as demonstrated by antibody prevalence in children less than 6 years old. Differences according to sex are still unclear;

- in the villages studied antibody prevalence seems to be associated with lower population densities, i.e. outlying areas versus centre of villages;

- the seropositivity rate is not equally distributed through the population. Some families have a high seropositivity rate (up to 50% or more), others have no seropositive member;

- concerning a possible animal reservoir, rodents, cattle, chicken, dogs can be infected.

Further studies are needed :

1. the specificity of filovirus serologic reaction must be studied, in order to know whose viral proteins are recognized by antibodies detected by immunofulorescence assay. It would be especially important to determine whether these antibodies are specific of Ebola or Marburg virus, and if not, whether they can neutralize an infection by these viruses. This work is only possible in a specialized laboratory;

2. obviously efforts should be made to isolate the viral agent detected by the filovirus serology. Isolating a weakly or non- pathogenic agent without a known animal reservoir will be probably difficult. Our current approach is to attempt virus isolations from suggestive clinical specimens obtained from different places in and out the Central African Republic;

3. continuous surveillance of human and animal populations is also necessary to establish risk factors and to understand the epidemiology of filovirus. This study would consist in spending a long time (1 or 2 months), with the assistance of an anthropologist and a mammalogist in order to make an inventory of possible contamination sources (animal and others), study the socio-cultural factors of transmission, evaluate the possibility of inter-human contamination.

4. Several hemorrhagic fever viruses have been demonstrated in CAR, clinically, virologically or serologically : Yellow fever virus, Rift Valley fever virus, Congo-Crimean hemorrhagic fever virus, to which we must add viruses whose pathogenicity has not been yet established : Ippy and Mobala viruses (Arenavirus) and a member of the filovirus group.
These viruses represent a permanent biohazard for human populations in CAR. Therefore a continuous surveillance of hemorrhagic fevers, including epidemiological, clinical and virological aspects, remains necessary.

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