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Ebola and Marburg virus antibody prevalence in selected populations of the Central African Republic

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ABSTRACT – With the natural history of the filovirus family seemingly unknown, filovirus ecology in its natural environment remains a rudimentary field of research. In order to investigate the maintenance cycle of filovirus in Central Africa, a study was conducted within the rain forest of the Central African Republic. The epidemiological study determines the frequency and distribution of filovirus seroprevalence in a selected human population. Using an ELISA, serum samples from Pygmy and non-Pygmy populations were tested for Ebola-Zaire virus and Marburg (MBG) virus antibody. Filovirus antibody reacting sera were found in all zones investigated, and in all populations studied (Ebola virus IgG 5.3%; Marburg virus IgG 2.4%). Pygmies appeared to have a significantly higher seroprevalence ($P < 0.03$) against Ebola-Zaire virus (7.02%) than non-Pygmies (4.2%). MBG virus or related unknown filovirus strains also seem to be present in the western part of Central Africa. MBG virus antibodies were present in different Pygmy groups (ranging from 0.7 to 5.6%, mean 2.05%) and in several non-Pygmy populations (ranging from 0.0 to 3.9%, mean 3.4%) without an overall significant difference between the two groups ($P = 0.14$). The potentialities of nonpathogenic filovirus strains circulating in the Central African Republic are discussed. © 2000 Éditions scientifiques et médicales Elsevier SAS

Ebola virus / Marburg virus / Central African Republic / serology / ecology

1. Introduction

Since the discovery of the filoviruses more than three decades ago, their natural maintenance cycle remains poorly defined. Ebola (EBO) virus epidemics occurred in Sudan in 1976, 1979 and 1995, in the Democratic Republic of the Congo (DRC, former Zaire) in 1976 and 1995 [1, 2], and in Gabon in 1994 to 1996 [3]. These countries shared the same history of human migration and contemporary lifestyle with their neighboring country, the Central African Republic (CAR). Up until now CAR has been spared, but it is exposed to EBO virus emergence: Nzara, the village and cotton factory where the first Sudanese EBO outbreak occurred, lies 150 km from the CAR border, in the same savanna-forest mosaic zone that covers one third of CAR [4]. Less than 150 km south of the Oubangui River, which is the natural border between northern DRC and CAR, are the villages of Yambuku and Tandala, where the first Zairian EBO infections took place [1, 5]. Both villages are part of the large phytogeographical zone of the Congolese rain forest that extensively covers the southern part of CAR. Cultural and environmental similarities, along with the proximity of previous EBO virus manifestations, favored the choice of CAR as a study site for filovirus ecology. Another factor of the choice was the similarity of CAR's ecological domain with that of Uganda, which was the country of origin of the monkeys exported to Europe in 1967 and the source of the first Marburg (MBG) virus outbreak and of the virus' isolation [6]. It is clear that EBO

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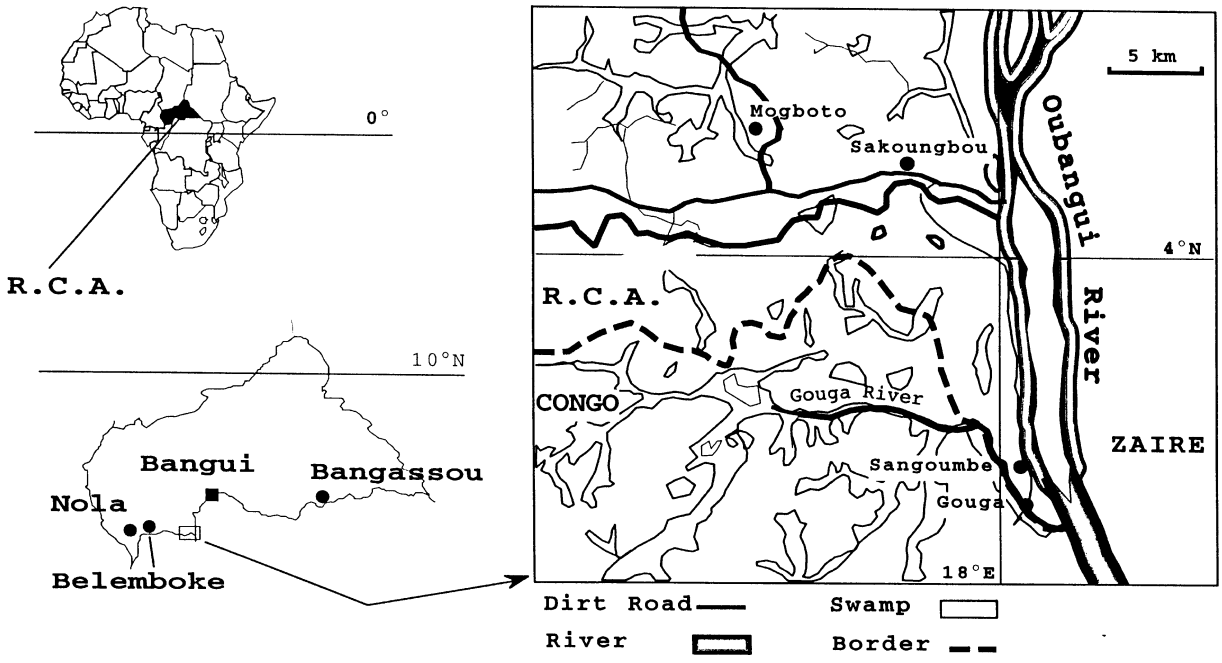

Figure 1. Map of the Oubangui and Lobaye River confluent showing the main study sites, Central African Republic. The map insets show the location of Lobaye District within the Central African Republic.

virus is present in CAR: as early as 1979, filovirus serological markers were detected by immunofluorescence antibody test and radioimmunoassay in the human population of CAR [7-10]. In the 1980s, several research programs on filovirus epidemiology were carried out in a collaborative effort between the Institut Pasteur, Bangui, the Centers for Disease Control, the United States Army Medical Research Institute for Infectious Diseases, in Fort Detrick and the Organisation de Coordination pour la lutte contre les grandes endémies en Afrique Centrale, Yaoundé [10-12].

In order to investigate the potential of active circulation of filovirus in CAR, study sites and study populations were identified in the Lobaye district, where high filovirus seroprevalence was previously detected [9, 12]. A three-phase strategy was then established for 1) determining the human population at risk of filovirus infection, 2) identifying the potential risks factors associated with subsistence activities such as hunting and gathering products from the forest, and 3) identifying potential reservoir hosts of the virus in nonprimates, rodents, bats and their ectoparasites. Here we report a first phase of an ongoing research program for filovirus ecology in CAR, and present our findings on an ELISA serosurvey principally of the Lobaye district's human populations as well as other pilot studies.

2. Materials and methods

2.1. Study area

A mixture of preforest grassland and Congolese rain forest covers the Lobaye district *(figure 1)*. It is a primary semi-deciduous, dense rain forest, which was long ago transformed by man, and is now partly exploited by man (logging, farming in clearings) and damaged along the roads and logging trails. The Pygmy camps (Sangoumbe, Sakoungbou and Mogboto) and non-Pygmy villages (Gouga village) we studied are located in a remote forest area (3°38 N, 18°03 E) 120 km south of Bangui, near the Oubangui River and south of one of its major tributaries, the Lobaye River. The Gouga villages are located about two km from the Oubangui River and the Congo's (Brazzaville) border.

The sera were collected in November 1995 at the beginning of the dry season, which lasts about four months.

In order to compare similar human populations and environments to that of Lobaye, four series of serum samples were also collected and tested. Sera were obtained from Belemboke (3°12 N, 16°15 E) in December 1992 and November 1994, and from Nola (3°52 N 16°08 E) in December 1995 – both located in the Dzanga-Sangha district, 400 km southwest of Bangui. The two locations are secondary forest areas degraded by agricultural activities. The other set of sera was collected in October 1996, in a non-Pygmy population from Bangassou (4°41 N, 22°48 E, Mbomou district), located 500 km east of Bangui. Bangassou belongs to the forested savanna, with forest galleries at the edge of the rain forest.

2.2. Study population

Residents of the study regions are for the most part either farmers belonging to Bantu, Banda, or Oubanguian language groups, or hunter-gatherers (Pygmies) called

BaAka group (or Aka). This ethnic group distribution extends across the border of CAR into the northern part of the Congo and partially into southeastern Cameroon [13].

In the Lobaye district, three groups of Pygmy Aka camps (Sangoumbe, Sakoungbou and Mogboto) and a village group (Gouga village) were investigated. Pygmy camps are seasonal (three to six months) but each campsite can be occupied for an interval throughout the year. Each primary camp is composed of three to five secondary camps units, each unit consisting of three to five primary families from the same lineage, living in huts made of leaves and lianas. Sangoumbe is located in a forest clearing, 3 km north of Gouga. Sakoungbou's camps are along a logging trail, near the Lobaye River. Mogboto is 10 km west of Sakoungbou in a forest clearing. Despite increasing contact with the villagers living in the forest, most of the Pygmies from that area have preserved a characteristic seminomadic lifestyle based on hunting and gathering rain forest natural resources. Villagers belong to Mbati tribe (Bantu-speaking) and the remainder to the Mbanza and Ngbundu tribes (Banda-speaking). Less than one kilometer away, the Gouga village is a typical village of the Central African forest: 34 primary families (384 individuals) living in mud huts covered with woven palm tree leaves. Dwellings are located on both sides of a narrow dirt road on the shaded rain forest. They practice subsistence farming (manioc, banana, coffee, yam), hunting (monkey, duiker, antelope) and limited trapping (rodent, hedgehog) and fishing.

In Belemboke and Nola zones, a sedentary Pygmy population living nearby in a missionary compound seemed to have shifted from hunter-gatherers to subsistence farmers. In the region of Bangassou, only the non-Pygmy population was sampled.

2.3. Blood collection

A 10-cm^3 blood specimen was obtained using vacutainer (Becton Dickinson, France) from each volunteer as previously described [9]. Blood was centrifuged on site and sera kept in cryovials in a liquid nitrogen tank. An interview form was completed which documented individual demographic data (name, estimated age, sex, parents, residence, ethnic group), occupation and means of subsistence.

2.4. Filovirus antigen preparation

ELISA antigens were prepared at the Institute für Virologie, in Marburg. Virus strains and the techniques were those in use at the Institute für Virologie and described in detail elsewhere [14, 15]. Briefly, EBO-Zaire virus (Mayinga strain) and MBG virus (Musoke strain) were grown in E6-Vero cells. Centrifugation and filtration (200-kDa filter) cleared supernatant from infected cell culture. Virus was then concentrated by precipitation and sucrose/tartrate gradient ultracentrifugation. The concentrated virus was then inactivated by B-propiolactone. The final product was sonicated and used as positive antigen after dilution in carbonate buffer for coating microplates. The control antigen, uninfected E6-Vero cell supernatant, was prepared following an identical procedure (centrifugation, B-propiolactone inactivation, and sonication).

2.5. ELISA

Tests were performed at the Institut Pasteur of Bangui using a direct ELISA test for immunoglobulin G (IgG) detection as described by Ksiazek [16]. Briefly: polystyrene microplaques were used (Immulon II, Dynatech Laboratories, Alexandria, VA USA) and directly coated at 4 °C overnight with a suspension of the EBO antigen and the control antigen. Sera were diluted 1:100 in 5% skim milk in 0.01 M phosphate-buffered saline with 0.5% Tween-20 and subsequently through 1:6 400 in fourfold dilution in microplates. Each sample was tested against EBO antigen and control antigen. Specific IgG binding was revealed by an antihuman immunoglobulin G (Kirkegaard and Perry, Gaithersburg, MD) conjugated to horseradish peroxydase. After adding a chromogenic substrate, the optical density was measured at 450 nm using a spectrophotometer (LP 2100, Sanofi Diagnostics Pasteur). Differential optical density (DOD) for each dilution was calculated by subtracting the optical density (OD) obtained with the control antigen from the OD of the EBO antigen. The cutoff value was defined by using a mean OD + 2 SD from known negative control sera. Any DOD greater than 0.300 was regarded as positive. Serum specimens were considered positive if their titer was ≥ 1:400 and the sum of the OD of all four dilutions was greater than 1.000.

3. Results

Serological evidence of EBO virus and MBG virus circulation was found in all zones investigated: 5.3% (71/1331) of the population were seropositive for EBO virus IgG antibodies, while only 2.4% (33/1340) were seroreactive with MBG virus antigen.

In the Lobaye district, EBO and MBG virus reacting antibodies were detected in each village and camp investigated *(tables I* and *II)*. Although the sample size was not always sufficient to obtain statistical significance, some trends were still observed. The Aka Pygmy population including the three camps of Sakoungbou, Sangoumbe and Mogboto consistently showed higher antibody prevalence against both EBO-Zaire (13.2%) and MBG (5.2%) viruses than the non-Pygmy villagers (respectively, 4.0 and 0.0%). EBO virus antibody prevalence observed among Pygmy females was higher (15.3%) than that of males (10.9%) but not statistically significant (Chi^2, $P = 0.15$). However, among Pygmies, EBO virus antibody prevalence was significantly higher among the 21–30 age group ($P < 0.05$) (data not shown). MBG virus antibodies were found exclusively in the Pygmy population (5.2%). Among the Pygmies, males were more likely to present a higher rate of MBG antibody prevalence (7.5%) than females (3.1%) ($P < 0.05$).

In the other investigated areas *(table III)*, a higher EBO virus antibody seroprevalence was also observed in the Pygmy population than in villagers. Pygmies living in Belemboke presented an increasing EBO virus antibody prevalence between 1992 and 1994 ($P = 0.003$) without clinical manifestation. In the non-Pygmy population living

Table I. Ethnic and sex distribution of Ebola virus reacting antibodies (IgG EBO, ELISA ≥ 1:400) in Lobaye district, CAR.

Ethnic group and location	*Total samples*	*Male*	*Female*	*Total (%)*
Pygmies				
Sangoumbe	51	2/23*	4/28	6(13.3)
Sakoungbou	48	4/24	4/24	8(16.6)
Mogboto	91	4/45	7/46	11(12.1)
Subtotal	190	10/92 (10.9)**	15/98 (15.3)	25(13.2)
Bantus				
Gouga	50	0/26	2/24	2(4.0)

* pos/total; ** sexe; $P = 0.15$.

in Nola and Bangassou, EBO virus seroprevalence was similar to that of the same population group of the Lobaye district. MBG antibody prevalence was higher in farmers than in the Pygmy group of Belemboke.

In this study, only 4/1331 serum samples had both EBO virus and MBG virus antibody *(table IV)*, suggesting dual infections. As a control, 68 coded serum samples (14 EBO virus reactive and 54 nonreactive) were sent to the Centers for Disease Control (Special Pathogens Branch, Dr T. Ksiazek), Atlanta to be expertised and tested against EBO-Zaire, EBO-Sudan, and EBO-Ivory Coast antigens. All results were confirmed, and only one serum sample was found double reacting EBO-Zaire-positive (1:1,600) and EBO-Sudan (1:400)-positive.

4. Discussion

Seroepidemiological studies help to understand the transmission cycle. A determining factor lies in the techniques used. The limitations of indirect immunofluorescent assay were recognized early [17]. ELISA technique for the detection of IgG EBO virus antibodies has recently been recommended by several authors [18, 19] and combined a better specificity and a high sensibility. In this study, we used EBO-Zaire as antigen in the ELISA technique and we have observed a very low rate of cross-reactivity with the three other subtypes of EBO virus and only 0.3% of reactive sera showed a dual positivity EBO-MBG. These findings suggest that EBO virus circulating in CAR is more likely a Central African EBO-Zaire-like virus.

From previous EBO virus clinical and serological surveys done in Central Africa [8–11] studying the geographical distribution of EBO virus infection [14, 15, 17], and the present data, it appears that EBO viruses circulated in Africa between the 1 500 isohyet north and south of the equator, which corresponds to the limits of the rain forest/forested savanna domain. The EBO virus strain from the Taï forest of Ivory Coast has been found in the same

Table II. Ethnic and sex distribution of MBG virus reacting antibodies (IgG MBG, ELISA ≥ 1:400) in Lobaye district, CAR.

Ethnic group and location	*N sera*	*Male*	*Female*	*Total (%)*
Pygmies				
Sangoumbe	52	2/24*	2/28	4(7.7)
Sakoungbou	48	1/24	0/24	1(2.1)
Mogboto	91	4/45	1/46	5(5.6)
Subtotal	191	7/92 (7.5)*	3/98 (3.1)	10(5.2)
Bantus				
Gouga	50	0/26	0/24	0(0.0)

* pos/total (percentage).

Table III. EBO virus and MBG virus antibody prevalence (ELISA ≥ 1:400) in inhabitants of Belemboke, Nola, and Bangassou.

Location	*EBO*	*MBG*		
	Pygmies	*Villagers*	*Pygmies*	*Villagers*
Belemboke 1992	7/361(1.9)*	–	3/361(0.8)	–
Belemboke 1994	16/132(12.1)	3/98(3.1)	1/130(0.7)	2/98(2.0)
Bangassou 1995	–	8/226(3.6)	–	9/236(3.9)
Nola 1995	–	10/274(3.7)	–	8/274(3.3)

* pos/total (percentage).

Table IV. Comparative antibody titer (ELISA) of EBO virus and MBG virus antigen dual reactive sera from the Lobaye district, CAR.

Location	*Ref sera*	*TiterEBO*	*Titer MBG*
Belemboke 1994	B33	6400	400
Nola 1995	229T08	400	400
Lobaye	LB33	1600	400
Lobaye	LB94	6400	400

ecological zone as its most closely genetically related EBO-Zaire strain [20]. The limited divergence between the two strains and their association within the same forested zone may suggest a common origin, since the Ivorian forested massif was at one time part of the Congolese rain forest from which it became separated during the last glaciation (16 000 years ago). Because of the phytogeographical composition of CAR and its relatedness to the emergence zones of EBO virus, this country is believed to host a seat of filovirus circulation.

Our present study done in human populations living in forested areas of CAR has bought to the fore two main observations: 1/ active circulation of filovirus without apparent clinical manifestations; 2/ potential association of filovirus infection and a specific lifestyle.

This study performed in forested areas showed that the Pygmy population of CAR living in camps appeared to be more at risk to filovirus infection than sedentary villagers. However no trend of seropositivity was observed for Pygmies living in camps in the deep forest compared to those living near a road, where local trade with villagers resulted in reduced contact with the forest for subsistence activities. Pygmies from Lobaye and Belemboke appeared to have the same seroprevalence over recent years. Each of the non-Pygmy sample population involved in subsistence farming and trading activities presented the same EBO virus antibody prevalence. One finding was the presence of significant MBG virus antibodies in the population of CAR. In accordance with previous findings [9, 14, 15], this unusually high antibody prevalence suggests a circulation of MBG virus or MBG-like virus within that part of Central Africa situated in the same ecological zone as the suspected Ugandese origin of the MBG virus. MBG virus seroprevalence was significantly higher ($P > 0.05$) for the Pygmy population of the Lobaye than for the one of Belemboke, a situation which remains unexplained.

The forest origin of the potential reservoir of EBO virus is supported by serosurvey among human populations, although epidemic outbreak occurred in the forest-savanna ecotone in Sudan, DRC and Gabon. If the risk is present in two different ecosystems, research for detection of the reservoir must be not limited to rain forest but investigations need to be extended to forest-savanna ecotone.

Clinical cases of EBO virus infection are rare and could suggest that risk of human infection from the host reservoir is low, but serologic results indicate that exposure to EBO virus is not uncommon and didn't always result in obvious human disease. Previous observations and studies using different laboratory techniques suggested that unknown filoviruses with variable pathogenicity might exist in different parts of the world [14]. The hypothesis of Monath [21] is that pathogenic strains have independent transmission cycles involving host species rarely in contact with humans, or they emerge from nonpathogenic strains by mutational events. Humans could select virulent strain from the reservoir. On the basis of rates of EBO virus antibody seroprevalence observed, the hypothesis of the circulation of nonpathogenic EBO virus strains seems to be the more favorable one. It is possible that the filoviruses represent a diverse complex of nonvirulent enzootic strains and virulent variants, which emerge from the enzootic cycle. Moreover, the existence of EBO-Reston and EBO-Ivory Coast viruses isolated from primates and highly pathogenic for chimpanzees but nonfatal for man (although the number of human infections is small), suggests that other filoviruses with suspected lower human pathogenicity exist and infect primates in the natural environment [14, 20]. During a five-year project on clinical epidemiological survey in CAR, neither clinical syndromes nor outbreaks mimicking either EBO virus or MBG virus infection where recorded. Moreover during a four-year serosurvey of a cohort of 200 persons conducted in CAR, in fourteen instances seroconversions against EBO-Zaire virus without clinical manifestation were detected (J.P. Gonzalez and E.D. Johnson, unpublished data).

Our observations suggest that populations of CAR have been in contact with EBO virus and MBG virus previously. EBO virus distribution in Central Africa appears limited to a distinct ecosystem geographically associated within the rain forest belt extending to forest-savanna ecotone. This observation will help to select animal collection sites to elucidate the EBO virus natural reservoir(s). Finally, epidemics seem to be related more to human behavior, which increases risk of contact with the reservoir than to the emergence of a highly pathogenic strain. However EBO virus strains circulate in the rain forest and, with man's intrusion, could led to infection. In a rare event, which has not been yet discovered, a highly pathogenic strain can strike and cause subsequent outbreak. Differences between communities living in forest regions, such as habitat, human activities, and agricultural practices, may modify the risk of infection.

MBG virus antibodies were present in different Pygmy groups with an antibody seroprevalence ranging from 0.7 to 5.6% (mean 2.05%), but also in several non-Pygmy populations (0.0 to 3.9%, mean 3.4%). No significant difference was recorded between the two groups ($P = 0.14$). MBG virus or related unknown filovirus strains also seem to be present in the western part of Central Africa and

infect humans. However it probably persists in a different selvatic cycle than EBO virus with respect to its apparent extension from Central to East Africa and a very low seroprevalence is encountered [9, 11].

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