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Filovirus activity among selected ethnic groups inhabiting the tropical forest of equatorial Africa

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Abstract

Seroepidemiological surveys were conducted to determine the frequency and distribution of filovirus activity among selected ethnic groups inhabiting the tropical forests of the Central African Republic. 427 serum specimens were collected from hunter-gatherers and subsistence farmers living in forest environs in the Lobaye District south of the river Lobaye and west of the river Oubangui. Striking serological evidence for filovirus activity was found in both populations. Ebola virus appears to be the most active filovirus; 17·6% (75/427) of the Lobaye survey population were seropositive for Ebola virus reactive antibody while 1·2% (5/427) were seroreactive with Marburg viral antigens. Ethnic background appeared to be an important risk factor influencing filovirus exposure in the forest communities. The filovirus antibody prevalence among 21–40 years old male Aka Pygmy hunter-gatherers was significantly ($P=0·03$) 3 times higher (37·5%) than that in similarly aged male Monzombo and Mbati subsistence farmers (13·2%). Continued epidemiological investigations are needed to define ethnic-related events influencing human filovirus activity in the Congo basin of equatorial Africa.

Introduction

The filoviruses Ebola and Marburg viruses have caused sporadic but widespread epidemics of fatal haemorrhagic disease in sub-Saharan Africa. These highly pathogenic viruses circulate undetected, presumably as enzootic infections, until conditions change favouring their expression and recognition as severe human pathogens (SIEGERT, 1970; SMITH, 1978).

A concerted effort was begun in 1984 to define risk factors for infections and the natural threat posed by the filoviruses in central Africa. Over the 4 year period 1984–1987 cross-sectional and prospective epidemiological and clinical surveillance studies were conducted in which serosurvey samples and clinical specimens from fever cases of unknown aetiology were assessed for evidence of haemorrhagic fever virus activity. Our early findings, suggesting frequent filovirus exposures in selected central African populations, have been presented elsewhere (GONZALEZ *et al.*, 1982, 1989; MEUNIER *et al.*, 1987).

This paper reports the frequency of filovirus activity, as measured by the prevalence of virus reactive antibody, among selected ethnic groups, Aka Pygmies or hunter-gatherers and Monzombo and Mbati villagers or subsistence farmers, inhabiting the Lobaye District forest environs of the Central African Republic. Potential epidemiological and ecological factors which may influence endemic virus activity are also discussed.

Materials and Methods

Survey populations

During the 1987 dry season, forest villages in the Mongoumba region of the Lobaye District were serosurveyed. Medical clinics were held in the Monzombo and Mbati villages Molabaye, Yabongo, Ikoumba, Saboulou, and in Gouga and Aka forest camps to assess the general health of the local forest populations. Physical injuries, burns, minor myalgic and arthritic disorders, bacterial, fungal and parasitic infections were treated. Village and forest residents were informed of the purpose of the serosurvey; volunteers were interviewed, serosurveyed, and issued a registration card containing the individual's name and identification number used to label corresponding specimens. An interview form was completed documenting the individual's name, identification number, estimated age, sex, village of residence, length of residence, ethnic group, occupation, father's name, mother's name, and husband's or wife's name.

Sample collection

Venous blood was drawn aseptically from the antecubital fossa following accepted standards governing procedures to be used with human subjects. The blood was allowed to clot overnight at 4°C. The serum was separated by centrifugation, dispensed into cryotubes and immediately frozen in liquid nitrogen for transport to the laboratory.

Immunofluorescent antibody test

An indirect immunofluorescent antibody test (IFAT) using monovalent and polyvalent spot slides of acetone-fixed virus and sham-infected cells was used throughout the study to detect and measure haemorrhagic fever virus (HFV) reactive antibody (JOHNSON, E. D. *et al.*, 1993). Monovalent slides were prepared using uninfected cells mixed 10:1 with cells infected with a single HFV. Polyvalent slides were produced using a mixture of equivalent numbers of sham-infected cells and cells infected with each of 6 African haemorrhagic fever viruses (AHFV) listed below.

The Mayinga strain (Zaire isolate) and Boniface strain (Sudan isolate) of Ebola virus (EBOV), the Musoki strain (Kenya isolate) of Marburg virus (MBGV), the Josiah strain (Sierra Leone isolate) of Lassa virus, the 10200 strain (Uganda isolate) of Crimean-Congo haemorrhagic fever virus (CCHFV), and the ZH501 strain (Egypt isolate) of Rift Valley fever virus (RVFV) were used to prepare serological reagents (JOHNSON, B. K. *et al.*, 1982; JOHNSON, E. D. *et al.*, 1993). Infectious HFV preparations were handled within the containment facilities designed and equipped for maximum biological containment (level 3 and 4) at the United States Army Medical Research Institute of Infectious Diseases.

The IFAT was standardized using convalescent plasma or immune serum from confirmed HFV infections and goat anti-human γ globulin conjugated with fluorescein isothiocyanate. Each test was performed double-blind. Questionable IFAT reactions were re-examined by a reviewer and technician and/or an outside observer. The tests were repeated when a consensus could not be reached concerning inconclusive reactions. The few sera which produced inconclusive reactions on retesting were considered non-specific IFAT reactors.

The survey specimens were screened for virus-reactive antibody at 1:16 dilution on polyvalent slides. Virus specificity was determined by rescreening the IFAT seroreactive specimens on monovalent slides. The level of seroreactivity was determined by titrating the IFAT reactions on monovalent slides using two-fold serial dilutions. Antibody titres were recorded as the reciprocal of the last clearly positive dilution. Specimens that reacted with infected but not uninfected monovalent cell preparations at 1:128 or greater dilution were considered positive for virus reactive antibody. The conservative,

high antibody titre, 1:128, was selected as the cut-off point between seropositives and seronegatives to reduce the likelihood of including false positives (JOHNSON, E. D. *et al.*, 1993).

Statistical analysis

The significance of differences between HFV antibody prevalence rates was determined by χ^2 analysis (SNEDECOR & COCHRAN, 1969).

Results

African haemorrhagic fever virus antibody prevalence

Evidence of AHVF activity was found in the Lobaye study population; 18·7% (80/427) of the population were seropositive for HFV, with titres ≥128. The filoviruses appeared to have been the most active AHFV in 1987: 17·5% (75/427) of inhabitants were filovirus seropositive; 0·7% (3/427) were RVFV seropositive; and 0·5% (2/427) were positive for Lassa virus antibody. Serological evidence for human CCHFV activity was not found.

Filovirus antibody prevalence

The majority of filovirus seroreactive specimens from Lobaye District (94·6%, 71/75) reacted with the EBOV strains and not with MBGV (GONZALEZ *et al.*, 1983). Sixty-one per cent (45/74) of the EBOV seropositive samples reacted with both EBOV-Zaire (EBOV-Z) and EBOV-Sudan (EBOV-S) viral antigens and were considered positive for EBOV group specific antibody. Broad 'family' reactions were observed in 4·1% (3/74) of the seropositive samples which reacted with both EBOV and MBVG antigens. Filovirus strain-specific responses (EBOV-Z, EBOV-S, or MBGV) were also observed; 8·0% (6/75), 30·7% (23/75) and 1·3% (1/75) of the total number positive were EBOV-Z, EBOV-S or MBGV seroreactive, respectively.

Table. Sex distribution of filovirus antibody seropositivity among hunter-gatherers and farmers in Lobaye District, Central African Republic

	No. seropositive[a]		
	Total	Male	Female
Hunter-gatherers	31/127 (24·4)	13/56 (23·2)	18/71 (25·3)
Farmers	42/300 (14·4)	18/169 (10·7)	24/131 (18·3)

[a]Number seropositive/total number tested (with percentages in parentheses).

Risk factors for filovirus infections

Ethnic background appeared to be an important factor influencing filovirus antibody activity among the forest communities (Table). The filovirus antibody prevalence in hunter-gatherers was significantly higher ($P=0·011$) than that among farmers. The antibody prevalence in both male and female forest dwellers was higher than the level found in corresponding male and female village residents; however, only the male difference was statistically significant ($P=0·025$).

Ethnic background remained an important factor when sex- and age-specific filovirus reactive antibody prevalence rates were compared (Figure). The antibody prevalence appeared to be 2 or 3 times higher among male hunter-gatherers than in similarly aged male farmers. However, the difference was statistically significant ($P=0·03$) in only the 21–40 years old male age group (Pygmies, 37·5%; farmers 13·2%). Differences between female Pygmy and farmer age-specific antibody prevalences were less striking. The antibody prevalence was higher among the 21–40 year old female hunter-gatherers (30·8%) than among similarly aged farmers (18·6%), but the difference was not statistically significant ($P=0·22$).

Discussion

Our results indicate that filovirus infections are common among inhabitants of the dense tropical forest of Lobaye District, Central African Republic. Virus activity, as measured by antibody prevalence, appears to be influenced by risk factors associated with the ethnic groups of the Congo basin forest. The infections appear to occur without significant overt disease or increased mortality, suggesting that African filoviruses may be less pathogenic in their natural environment than expected, or that filovirus infections are unrecognized, being confused with some common local illness or culturally defined disease occurring in the Lobaye forest.

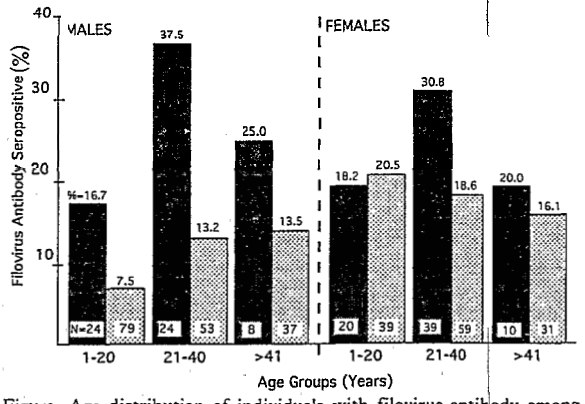

Figure. Age distribution of individuals with filovirus antibody among hunter-gatherers (dark blocks) and farmers (lighter blocks) in Lobaye District, Central African Republic.

The surveyed population of 427 forest inhabitants consisted of 400 members of 3 ethnic groups, 127 Aka, 219 Monzombo, and 54 Mbati, and 27 individuals whose ethnic background was not determined. These groups share the forest resources south of the Lobaye river and west of the Oubangui river but have different life styles. The Aka from Mongoumba region are nomadic hunter-gatherers who move 5 to 6 times a year, establishing camps within a defined territory without cultivating crops (BAHUCHET, 1990). The Monzombo and Mbati are sedentary, riverine farmers who practice slash-and-burn, shifting cultivation (BAHUCHET *et al.*, 1990). Though each of these populations follows a distinct lifestyle, their forest subsistence often depends upon a mutually beneficial and barter-based co-operative relationship (BAHUCHET, 1988). Forest farmers frequently trade cultivated yams and cassava for highly prized fresh game meat supplied by the Pygmies (BAHUCHET, 1988; BAILEY & PEACOCK, 1988; BAHUCHET *et al.*, 1990).

The difference in high titre filovirus antibody prevalences between adult male and female Aka forest dwellers strongly suggests that filovirus exposure in the Lobaye forest is influenced by factors associated with the hunter-gatherer subsistence strategy. The previously reported findings that the filovirus antibody prevalence is higher among Pygmy hunter-gatherers than Bantu subsistence farmers of the Lombie region of Cameroon support this interpretation (BAUREE & BERGMANN, 1983).

The relatively high antibody prevalence among male and female Pygmies was surprising. Based on seroepidemiological results, filovirus infections have been presumed to be a potential risk to female farmers; filovirus antibody prevalence rates were consistently higher in female than in similarly aged male subsistence farmers (JOHNSON, K. M., 1978; HAYMANN *et al.*, 1980). An analogous trend was observed among Monzombo and Mbati farmers living along the Oubangui river. The difference between antibody prevalences in males and females was notable in the 1–20 years age group. A sex-related difference in antibody prevalence might be expected since Monzombo and, presumably, Mbati females and males perform distinct subsistence tasks. Monzombo and Mbati females maintain multiple forest

garden plantations throughout the year, while males tend to be fishermen, often spending long periods in fishing camps along the river (BAHUCHET, 1988).

Collectively, our findings suggest the filoviruses are encountered during their daily occupation by a distinct subpopulation of male and female hunter-gatherers and female farmers inhabiting the eastern Lobaye. The putative risk factors may be related to the collection and handling of meat. The forest provides the bulk of the animal protein consumed by Lobaye residents. Large mammals like antelope and bush-pigs are netted or trapped during the dry season by Aka hunting bands consisting of both males and females while smaller animals such as monkeys and fruit bats are stalked by solitary male hunters during the August and September rainy season. Females prepare and distribute the best meat among the hunting band and often use the remainder to barter with village women (HART, 1978).

The consumption of *Cercopithecus* monkey meat may involve important risk factors for filovirus infection. Feral monkeys from diverse African habitats have been shown to be seropositive for filovirus antibody (JOHNSON, B. K. *et al.*, 1982; MATHIOT *et al.*, 1990); seronegative monkeys have been shown to be susceptible to laboratory filovirus infection but resistant to fatal disease (P. B. Jahrling, personal communication); wild-caught monkeys have been associated with two large filovirus outbreaks (HENDERSON *et al.*, 1971; JARHLING *et al.*, 1990); and the August 1976 EBOV haemorrhagic fever index case in Zaire had purchased fresh monkey meat 10 d before becoming fatally ill (JOHNSON, K. M., 1978). Monkeys, however, may not play a central role in the natural filovirus cycle; they may live in close association with the primary maintenance reservoir and only occasionally become infected. Filovirus transmission, therefore, may occur only in human populations whose subsistence strategy places them in frequent and prolonged contact with freshly killed animals.

An association between filovirus antibody and contact with feral monkeys among forest inhabitants may be unique to distinct forest groups. The antibody prevalence found in 1987 Lobaye hunter-gatherers (31/127) was roughly 3 times higher than that observed in 1984 serosurveys of Pygmies living in the Sangha district (9/121) of south-western Central African Republic ($P=0{\cdot}01$) (E. D. Johnson & J. P. Gonzalez, unpublished observations). A difference in filovirus antibody prevalences was also observed between Lodorodorf-Bipindi and Lombie Pygmies of Cameroon (BAUREE & BERGMANN, 1983). These differences may be the consequence of variations in environment or culture. Subsistence methods and, presumably, the importance of monkey meat vary among Pygmy groups of central Africa; some hunter-gatherer bands have given up their nomadic forest life style to become forest edge cultivators (HEWLETT *et al.*, 1986; BAHUCHET, 1990; BAHUCHET *et al.*, 1990).

Nevertheless, the hypothetical association between filovirus reactive antibody and feral monkeys should be explored by epidemiological and anthropological investigations, which may resolve the paradox of high antibody prevalences for the highly pathogenic filoviruses without notable disease; the prevalence of filovirus antibody may be high among survivors of a common disease associated with contact with feral monkeys in the tropical forest of equatorial Africa.

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