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 Ouhampe. 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 Mbatasi subsistence farmers (12.5%). 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; MEIJNTER 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 Mbatasi 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 Mangouba region of the Lobaye District were serosurveyed. Medical clinics were held in the Monzombo and Mbatasi villages Mobabaye, Yabongo, Isoumba, 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) was utilized to screen for the presence of antibody against Ebola and Marburg virus. Monovalent slides were prepared using infected but not uninfected monovalent cell preparations of each of 6 African haemorrhagic fever viruses (AHFV).

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 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.

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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 χ² analysis (SNEDCOR & 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 MBGV antigens. Filovirus strain-specific responses (EBOV-Z, EBOV-S, or MBGV) were also observed; 0-0% (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**

<table>
<thead>
<tr>
<th></th>
<th>No. seropositive*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hunter-gatherers</td>
<td>31/127 (24.4)</td>
</tr>
<tr>
<td>Farmers</td>
<td>42/200 (15.4)</td>
</tr>
<tr>
<td></td>
<td>Male</td>
</tr>
</tbody>
</table>

*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 both female 'family' groups was significantly lower (P = 0.011) than among males. 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 = 0.025) in only the 21-40 years old age group. The antibody prevalence was higher among the 21-40 year old female hunter-gatherers (30.8%) than among similarly aged females (16.6%), 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.

The surveyed population of 427 forest inhabitants consisted of 400 members of ethnic groups, 127 Aka, 219 Monzombo, and 54 Mbat, 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 lifestyle styles. The Aka from Mongouma region are nomadic hunter-gatherers who move 5 to 6 times a year, establishing camps within a defined territory without cultivating crops (BAHUCHET et al., 1990). The Monzombo and Mbat 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 yam 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 (BAUER & 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 females than in similarly aged male subsistence farmers (JOHNSON, K. M., 1978; HAMANN et al., 1980). An analogous trend was observed among Monzombo and Mbat 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, Mbat females and males perform distinct subsistence tasks. Monzombo and Mbat 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 (Bahuochet, 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 meat among the hunting band and often use the remains of 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 Ceropithecus 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 (John- son, B. K. et al., 1982; Mathi 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 (Jahrling 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 greater than that observed in 1984 serosurveys of Pygmies living in the Sangha district (9/212) of south-western Central African Republic (P = 0.01) (E. D. Johnson & J. P. Gonzalez, unpublished observations). A difference in filovirus antibody prevalences was also observed between Lodorodorf-Bipindi and Lombiye Pygmies of Cameroon (Baurre & 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; Bahuochet, 1990; Bahuochet 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.

References


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