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Investigation of a possible yellow fever epidemic and serosurvey for flavivirus infections in northern Cameroon, 1984

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A cluster of fatal hepatitis cases in northern Cameroon in 1984 stimulated a field investigation to rule out an epidemic of yellow fever. A serosurvey of villages in the extreme north of the country, in a Sudan savanna (SS) phytogeographical zone, disclosed no evidence of recent yellow fever infection. However, further south, in a Guinea savanna (GS) phytogeographical zone, serological evidence was found of endemic yellow fever virus transmission. The results indicate a potential for epidemic spread of yellow fever virus from the southern GS zone to the nothern SS zone of Cameroon, where immunity in the population was low.

In April 1984, the Ministry of Health of Cameroon and the WHO Regional Office for Africa, Brazzaville, requested the assistance of the Centers for Disease Control in ascertaining whether yellow fever was the cause of a cluster of fatal cases of hepatitis in the northern province of the country and whether a larger outbreak had occurred there. The index case was a pregnant woman who died of hepatitis in January 1984 in Maroua, a city in the sub-Sahelian northern province (see Fig. 1). Two more fatal cases of hepatitis among pregnant women were reported in January 1984 in the same city. A histopathological diagnosis of yellow fever was made in the index case, but in no instance was a specific diagnosis of yellow fever confirmed. Entomological surveys in 1981 and 1983 found that, during the rainy season, the extreme north of Cameroon was extensively infested with *Aedes aegypti* as well as with the sylvatic vectors *A. furcifer-taylori* and *A. vittatus* (M. Germain, unpublished report, 1981; R. Cordellier, unpublished report, 1983). However, these surveys and a survey in Maroua in 1984, which included the dwellings of two of the fatal hepatitis cases, found no evidence for *A. aegypti* in the northern province during the dry season (J. P. Adams, unpublished report, 1984).

We report here the results of our investigation to determine whether an outbreak of yellow fever had recently occurred in the north of Cameroon and whether conditions there were suitable for maintenance of yellow fever virus with the potential for epidemic spread to urban centres.

METHODS

It was not possible to trace and confirm the diagnosis of yellow fever in surviving patients; therefore, our efforts focused on serological surveys. A mass immunization campaign against yellow fever had last been performed in Cameroon in 1971. Consequently, the serosurveys focused on children aged of 13 years or less, who would have been born after the campaign. Samples of blood were taken from volunteers in five villages near Maroua in the northern, dry (Sudan) savanna (SS) phytogeographical

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zone of the country (Fig. 1). To establish whether sylvatic transmission of yellow fever might be prevalent among monkeys in the area, we also collected blood from *Cercopithecus aethiops* and *Erythrocebus patas* monkeys caught near Kossa. Further serosurveys were conducted in villages approximately 250 km further south (Poli, Fignole, and Gode), which lie in the moist (Guinea) savanna (GS) phytogeographical zone, corresponding to emergent zones of yellow fever elsewhere in West Africa (*1*). Although the disease had not been reported from this area, the moist savanna is capable of supporting high densities of vectors, suggesting that yellow fever might be enzootic and endemic in monkeys and humans, respectively.

Sera were tested by IgM capture enzyme immunoassay, haemagglutination inhibition (HI), complement-fixation (CF), and neutralization tests for antibody to yellow fever and selected other flaviviruses (Table 1) (*2*, *3*). Approximately 300 sera were tested in the field by enzyme immunoassay. None of the sera had IgM antibody to any of the flaviviruses tested, which was not unexpected, since the half-life of IgM antibody is short and sera were collected nearly 6 months after the rainy season, when the most recent infections would have occurred. The interpretation of the HI, CF, and neutralization antibody responses was based upon a previously published scheme developed to differentiate primary from flavivirus superinfections (*4*). Type-specific responses were classified as either monotypic, if antibody to a single antigen was detected, or homotypic, if heterologous antibody titres were four times lower than the homologous response.

Table 1. Schedule for the serological survey in Cameroon, 1984

1. All sera were screened by enzyme immunoassay for IgM antibodies to the following viruses: yellow fever, West Nile, and either Murray Valley encephalitis or Koutango.

2. All sera were screened by haemagglutination-inhibition (HI) for antibodies to yellow fever, West Nile, and Zika viruses. Sera with HI titres $\geq$1:80 to any antigen were:
   - tested further by complement fixation (CF) for antibodies to yellow fever, West Nile, Zika, Sepik, and Uganda S viruses, as well as for antibody to dengue virus type 2; and
   - screened for neutralizing antibody to yellow fever virus by plaque reduction neutralization (90% reduction was taken as the endpoint) in Vero cells. Sera with yellow fever virus neutralizing titres $\geq$1:16 were further tested for neutralizing antibodies to dengue virus type 2 as well as to Bouboui, West Nile, Zika, Sepik, and Uganda S viruses (selected sera) to determine specificity.

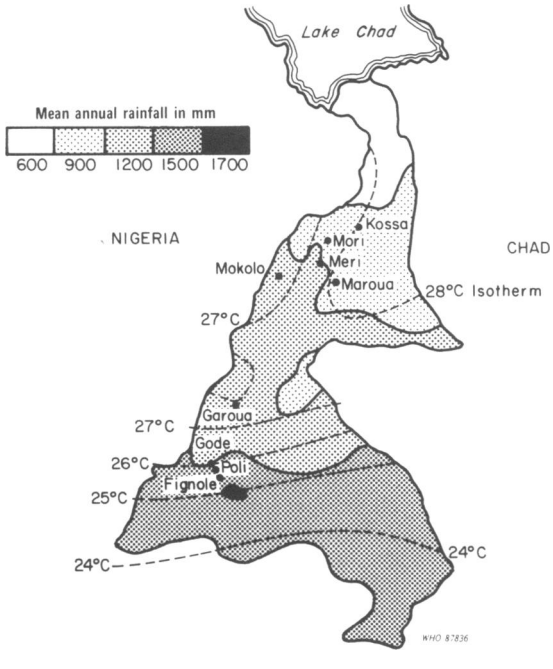

Fig. 1. Northern region of Cameroon, showing the sites of yellow fever investigation, 1984.

RESULTS

The results of the HI screening test showed that flavivirus infections were more prevalent in the northern SS zone than in the southern GS zone. Approximately 70–80% of persons in the SS zone had antibodies to yellow fever, West Nile, or Zika viruses, while in 70–80% of persons in the GS zone no antibodies to these antigens were detected in the HI test (Table 2).

On first appearances, the prevalence of yellow fever antibody seems high in the northern zone. However, stratification of the results indicates that only 9% of the participants in the north with positive seroresponses were monotypic, while a significantly greater proportion (34%) of responses to yellow fever virus in the south were monotypic (Table 3). These observations indicate that flavivirus superinfections were responsible for the apparent high prevalence of antibodies to yellow fever virus detected in the HI test in the north but that primary infection with the virus was more prevalent in the south.

The results of CF tests confirmed these indications (Table 4). Again, taking monotypic responses as

Table 2. Distribution of reciprocal titres in the haemagglutination-inhibition (HI) test for selected flavivirus antibodies by phytogeographical zone, Cameroon, 1984

Reciprocal HI titre	No. of sera					
	Yellow fever virus		West Nile virus		Zika virus	
	SS zone[a]	GS zone[b]	SS zone	GS zone	SS zone	GS zone
<10	94 (16)[c]	182 (72)	141 (24)	201 (79)	204 (35)	209 (82)
10	85 (14)	21 (8)	109 (19)	34 (13)	144 (25)	23 (9)
20	72 (12)	12 (5)	105 (18)	10 (4)	69 (12)	8 (3)
40	59 (10)	13 (5)	89 (15)	5 (2)	47 (8)	6 (2)
⩾80	276 (47)	26 (10)	143 (24)	4 (2)	122 (21)	8 (3)
Total	586 (99)	254 (100)	586 (100)	254 (100)	586 (101)	254 (99)

[a] SS = Sudan savanna.
[b] GS = Guinea savanna.
[c] Figures in parentheses are percentages.

specific indication of infection with yellow fever virus, we found little evidence that the virus had recently been active in the SS zone; however, recent infection may have occurred in as many as 2.4% of the population of the GS zone.

In contrast, West Nile virus infections were prevalent in the northern zone, where more than 9% of the population sample had evidence of recent infection with the virus (monotypic CF antibody).

The results of the neutralization tests confirmed the paucity of yellow fever activity in the northern SS zone (Table 5). Of 90 sera tested against yellow fever

Table 3. Response pattern in the haemagglutination-inhibition (HI) antibody test for selected flaviviruses by phytogeographical zone, Cameroon, 1984

Zone	No. of sera[a]		
	Yellow fever virus	West Nile virus	Zika virus
Sudan savanna	43/492 (9)	16/446 (4)	0/382
Guinea savanna	24/70 (34)	12/53 (23)	6/45 (13)

[a] Shown is the monotypic HI response/all HI responses. Figures in parentheses are percentages.

Table 4. Response pattern in the complement-fixation (CF) antibody test for selected flaviviruses by phytogeographical zone, Cameroon, 1984

Zone	No. of sera[a]					
	Yellow fever virus	West Nile virus	Sepik virus	DEN-2[b]	Uganda S virus	Zika virus
Sudan savanna	4/50 (0.7)	55/129 (9.4)	2/51	10/105	5/75	0/37
Guinea savanna	6/11 (2.4)	4/6 (1.6)	1/5	2/4	1/3	0/2

[a] Shown is monotypic CF response/all CF responses. Figures in parentheses are the estimated point prevalences of monotypic antibody in the population sample: Sudan savanna zone, $n = 586$; Guinea savanna zone, $n = 254$.
[b] DEN-2 = dengue virus type 2.

Table 5. Distribution of neutralizing antibodies for selected flaviviruses by phytogeographical zone, Cameroon, 1984

Zone	No. of sera[a]						
	Yellow fever virus	West Nile virus	Bouboui virus	Zika virus	DEN-2/Sepik virus[b]	Indeterminate	Total
Sudan savanna	10 (1.7)	47 (8.0)	0	1	0	32	90
Guinea savanna	17 (6.7)	2 (0.8)	2	0	0	3	24

[a] Shown are monotypic and homotypic responses. Figures in parentheses are the estimated point prevalences of specific antibody in the population sample: Sudan savanna zone, $n = 586$; Guinea savanna zone, $n = 254$.

[b] DEN-2 = dengue virus type 2.

and other flaviviruses, homotypic responses to the yellow fever virus were found in only 10 persons from the SS zone—all of whom were either over 14 years of age, who may have been immunized, or students at a secondary school, whose ages may not have been reliably given. In the GS zone, 17 persons had type-specific antibody to yellow fever virus. The median age of those who had positive seroresponses in the latter zone was 9 years, while in the north it was 12 years. The point prevalence of yellow fever viral antibody in the SS zone (1.7%) was significantly lower than that in the GS zone (6.7%) ($P<0.001$, Poisson).

The serosurvey did not aim to establish the prevalence of West Nile viral infections (sera were selected for neutralization tests on the basis of yellow fever virus neutralizing antibody titres of $\geqslant 1:16$, Table 1). However, of the sera tested, 47 from the SS zone had homotypic neutralizing antibody to West Nile virus, which is equivalent to an estimated point prevalence of 8%, and is significantly higher than the estimated prevalence in the GS zone ($P<0.001$, Poisson).

The serological survey of monkeys caught near Kossa established that sylvatic yellow fever did not occur in the SS zone and confirmed that West Nile

Table 6. Reciprocal titres of neutralizing antibodies for selected flaviviruses in monkeys, Kossa (Sudan savanna zone), Cameroon, 1984

Species	Reference number	Reciprocal titre					
		Yellow fever virus	West Nile virus	Zika virus	Uganda S virus	DEN-2[a] virus	Bouboui virus
Cercopithecus aethiops	75136	16	128	32	<4	<4	NA[b]
	75139	4	64	<4	NA	64	32
Erythrocebus patas	75140	4	256	<4	NA	32	16
	75141	4	256	<4	NA	<16	16
	75142	4	128	<4	NA	<16	<16
	75143	4	64	<4	NA	32	16
	75144	<4	NA	NA	NA	NA	NA
	75145	4	256	<4	NA	64	16
	75146	8	256	<4	NA	64	16
	75147	<4	NA	NA	NA	NA	NA
	75148	4	256	<4	NA	64	16
	75149	32	256	<4	<4	<4	NA
	75150	4	128	<4	NA	16	<16
	75151	8	64	<4	NA	32	<16
	75152	8	256	<4	NA	32	<16

[a] DEN-2 = dengue virus type 2.

[b] NA = Not available.

virus was the predominant flavivirus infection in the north of Cameroon (Table 6). None of the 15 monkeys tested had evidence of infection with yellow fever virus, and all 11 of the 13 monkeys that were tested for antibody to other flaviviruses had homotypic responses to West Nile virus.

DISCUSSION

The serological surveys reported indicate that yellow fever was not as widespread in the north of Cameroon as would have been expected had an outbreak recently occurred there. Further evidence against the occurrence of yellow fever in this part of the country came from histopathological reviews of the liver sections from the index case carried out independently by three consultants, one each at the Institut Pasteur, Paris; the Armed Forces Institute of Pathology, Washington, DC; and the Centers for Disease Control, Atlanta, GA. The consultants concluded unanimously that massive hepatic necrosis, consistent with viral hepatitis, was present. However, consultants A and B pointed out that microvesicular changes in the hepatocytes, the absence of Councilman's bodies, and the presence of an inflammatory infiltrate were features of the case that were atypical of yellow fever and indicated another viral etiology. Consultant C reserved judgment but agreed that the histopathology was not diagnostic of yellow fever. The limitations of diagnosing yellow fever based on histopathological findings alone are underscored by the discrepancies in the experts' diagnoses with the diagnosis of yellow fever made earlier in Yaoundé, which led to the investigation.

The combination of serological data, the results of entomological surveys, and the consultants' histopathological diagnoses indicate that yellow fever was not endemic in the northern province of Cameroon in early 1984. The serosurvey of monkeys confirmed the absence of sylvatic yellow fever in the SS zone. However, in the more southerly GS zone, serosurveys disclosed endemic transmission of yellow fever virus, as suspected. Therefore, conditions exist in Cameroon for maintenance of yellow fever virus in endemic areas, with the possibility of its emergence and introduction to susceptible populations in the north, resulting in subsequent epidemic spread.

We recommend improvements in the existing surveillance programmes for yellow fever in Cameroon and establishment of local laboratory facilities that would facilitate a timely, specific diagnosis of the disease in the country. The continuation of limited immunization campaigns in the north of the country and the inclusion of yellow fever immunization in the expanded programme on immunization are control measures being considered by the Ministry of Public Health.

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RÉSUMÉ

ÉTUDE SUR UNE ÉVENTUELLE ÉPIDÉMIE DE FIÈVRE JAUNE ET ENQUÊTE SÉROLOGIQUE SUR LES INFECTIONS À FLAVIVIRUS DANS LE NORD DU CAMEROUN, 1984

En 1984, plusieurs cas mortels d'hépatite survenus dans le nord du Cameroun ont conduit à entreprendre une étude de terrain afin d'écarter l'hypothèse d'une épidémie de fièvre jaune. Des enquêtes sérologiques ont été réalisées dans des villages de l'extrême nord du pays (zone phytogéographique de savane soudanaise) et dans des villages situés 250 km plus au sud (zone phytogéographique de savane guinéenne).

L'enquête a été axée sur les enfants de moins de 13 ans, nés après la dernière campagne nationale de vaccination de masse contre la fièvre jaune. Elle n'a montré aucun signe de transmission récente de la fièvre jaune dans la zone de savane soudanaise, ce qui a permis d'exclure l'hypothèse d'une épidémie. Dans cette zone, moins de 2% de la population présentaient des anticorps neutralisants contre la fièvre jaune, et 15 singes capturés aux alentours des villages d'étude n'ont montré aucun signe sérologique d'infection par le virus de la fièvre jaune, ce qui semble confirmer l'absence de transmission endémique et enzootique de la fièvre jaune dans cette zone. Toutefois,

dans l'échantillon de population observé, 8% des enfants présentaient des anticorps dirigés contre le virus West Nile.

Dans le sud, c'est-à-dire en zone de savane guinéenne, on a trouvé des signes d'endémicité de la fièvre jaune: des infections récentes, confirmées par la présence d'anticorps fixant le complément, ont été décelées chez 2,4% des enfants et 6,7% d'entre eux possédaient des anticorps neutralisants contre le virus de la fièvre jaune.

Les résultats de ces études montrent qu'il n'y a pas eu de transmission récente du virus de la fièvre jaune dans le nord, c'est-à-dire en zone de savane soudanaise, et que cette région ne connaît pas de transmission endémique du virus. La population de cette zone est donc sensible à l'infection et une épidémie se déclencherait si le virus était introduit. En revanche, la transmission du virus de la fièvre jaune est endémique dans le sud, en zone de savane guinéenne. Il existe ainsi un foyer d'activité permanente de la fièvre jaune, à partir duquel le virus peut se propager, à 250 km seulement des populations urbaines sensibles du nord.

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