

POXVIRUS IN WEST AFRICAN NONHUMAN PRIMATES:

SEROLOGIC SURVEY RESULTS¹

J.G. BREMAN²

J. BERNADOU³

J.H. NAKANO⁴

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2. Medical Epidemiologist, Bureau of Smallpox Eradication (BSE), Center for Disease Control (CDC), Atlanta, Georgia 30333, USA, attached to the Organization for the Coordination and Cooperation of Endemic Disease Control (Organisation de Coordination et de Coopération pour la lutte contre les Grandes Endémies (OCCGE)), Bobo-Dioulasso, Upper Volta.
3. Technical Officer, Office for Overseas Scientific and Technical Research (Office de la Recherche Scientifique et Technique d'Outre-Mer (ORSTOM)), Entomology Laboratory, Centre Muraz, OCCGE, Bobo-Dioulasso, Upper Volta.
4. Chief, Viral Exanthems Branch, Virology Division, Bureau of Laboratories, CDC, Atlanta, Georgia 30333, USA.

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
CENTER FOR DISEASE CONTROL
ATLANTA, GEORGIA 30333

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ABSTRACT

Poxvirus in West African Nonhuman Primates: Serologic Survey Results.
J.G. Breman, J. Bernadou, J.H. Nakano (Center for Disease Control,
Atlanta, Ga. 30333, Organisation de Coordination et de Coopération
pour la lutte contre les Grandes Endémies (OCCGE), BP 153, Bobo-Dioulasso,
Upper Volta, Office de la Recherche Scientifique et Technique d'Outre-Mer
(ORSTOM), BP 153, Bobo-Dioulasso, Upper Volta).

Ten species of nonhuman primates in West African habitat were analyzed for variola-vaccinia subgroup hemagglutination-inhibition (HI) and neutralization (neut) antibodies. The animals were taken in 27 different sampling zones in parts of the Ivory Coast, Mali, and Upper Volta. Eight % (15 of 195 tested) had elevated HI antibodies after nonspecific reactions were reduced with KIO_4 pretreatment. Positive neut antibodies were found in 21% (44 of 206). Antibodies were detected in serum from monkeys living near 2 areas where monkeypox cases in humans have occurred. Four samples were tested for monkeypox specific antibodies using an indirect immunofluorescent (IFA) test; 3 were positive. Despite the significant prevalence of poxvirus antibodies in monkeys (and other animals) in West Africa, smallpox eradication has been maintained in the area since 1970; thus, animal reservoirs of poxvirus appear to pose no threat to the worldwide smallpox eradication program.

INTRODUCTION

Monkeypox virus was first isolated in 1958, from captive Cynomolgus monkeys with exanthematous disease in Denmark (1). Nine subsequent outbreaks have occurred in nonhuman primate populations in the USA, The Netherlands and France (2,3). Since monkeypox was identified in humans in the tropical rain forest of the Republic of Zaire in 1970 (4) and in 4 other countries of West Africa in 1971 (5,6), various attempts have been made to link a nonhuman primate reservoir to human cases.

Serum samples from 2242 monkeys of several species from various parts of Africa and Asia were tested by different laboratories for neutralizing (neut) antibodies to variola-vaccinia antigen (7). No serum was felt to contain significant neut antibody. A survey of 100 Indian rhesus monkeys failed to detect either hemagglutination-inhibition (HI) or neut poxvirus antibodies (8). Other studies have shown significant variola-vaccinia group neut antibodies in serum of monkeys associated with humans with smallpox (9) and in the vicinity of humans with monkeypox (6,10,11).

To define possible nonhuman primate reservoirs of poxviruses in West Africa, we carried out surveys in areas where these primates were known to live naturally. These areas of West Africa are within the general geographic zone where human monkeypox cases have occurred (5,6).

METHODS

The study used serum samples collected in a study to define the presence of yellow fever virus circulating in monkey populations in member countries of the Organization for the Coordination and Cooperation of Endemic Disease Control (Organisation de Coordination et de Coopération pour la lutte contre les Grandes Endémies) (OCCGE)* between January, 1973, and May, 1974.

A map of West Africa was divided into degrees by parallels (latitudes) and meridians (longitudes) (fig. 1). A hunter (J.B.) was employed to shoot wild primates in 1 or 2 square degrees per month. Each square degree contains approximately 12,100 km². Primates were shot in 27 square degrees comprising about 327,000 km², containing parts of the Ivory Coast, Mali, and Upper Volta. Some zones bordered Ghana, Guinea, and Liberia. Primates were found in 2 major bio-climatic zones, the forest and heavily wooded preforest (5°N - 8°N) and the savanna (8°N - 15°N).

Immediately after shooting a monkey the hunter identified and inspected the animal for age (young, young adult, adult, old), sex, and gross superficial lesions. He drew blood by aseptic heart puncture. This was allowed to clot under refrigeration for less than 24 hours. Serum was centrifuged and then poured into a sterilized glass vial

* Benin (formerly Dahomey), Ivory Coast, Mali, Mauritania, Niger, Senegal, Togo, Upper Volta.

containing 4 to 5 drops of a solution containing penicillin, streptomycin, colymycin, and kanamycin. Serum samples were frozen at -20°C , packed in dry ice, and sent by air to the Center for Disease Control for testing.

HI antibodies to vaccinia antigen were measured at the Viral Exanthems Branch, Virology Division, CDC, by the microtiter technique of Hierholzer and Suggs (12,13) and neut antibodies by a plaque reduction method (14). An HI antibody titer of >10 (reciprocal of the dilution) and a neut antibody titer of >4 were considered positive. If the HI test was positive or enough serum was available, the test was repeated after the serum was treated with potassium periodate (KIO_4) to rule out a nonspecific reaction (15).

A subsample of serum that was HI- and/or neut-positive was tested for monkeypox antibody with an indirect immunofluorescence (IFA) test (16).

RESULTS

Two hundred forty-six primates from 10 savanna- and forest-dwelling species were shot (table 1); 56% of these were savanna-dwelling African green monkeys (Cercopithecus aethiops). No animal was noted to have external lesions consistent with a poxvirus disease..

Serum from 206 animals was tested for HI and neutralizing antibodies (table 2); 43% (89/206) had HI antibodies, but only 8% (15/195) were positive after KIO_4 pretreatment. Fourteen of 15 HI-positive serum samples were from Cercopithecus monkeys. Eleven of the HI-positive serum samples were from forest-dwelling monkeys. Monkeys were about equally divided by sex. Female monkeys with significant HI-antibody levels predominated over males, 11 to 4 (table 3); 21% (44/206) of the primates had detectable neut antibodies. Animals with neut antibodies were equally divided between sexes. Among young monkeys only 11% had neut antibodies, whereas 27% of adults had neut antibodies. No sex-related differences in neut antibody prevalence was observed.

Figure 2 indicates the distribution of the titers of animals with positive neut antibodies. Colobus monkeys had higher neut titers than Cercopithecus monkeys. Younger monkeys, if positive, tended to have higher neut titers.

Four specimens were tested for specific monkeypox antibodies. Three were positive (table 4). These 3 animals were found in zones within 200 km. of areas where human monkeypox cases have occurred.

The geographic distribution of monkeys with positive antibodies is shown in fig. 1. The highest concentration of positive animals was in heavily wooded savanna and the forest.

DISCUSSION

These serologic surveys indicate that both savanna- and forest-dwelling Cercopithecus and forest-dwelling Colobus monkeys in West Africa have been infected with a poxvirus, probably an Orthopoxvirus, the subgroup containing variola, vaccinia, monkeypox and whitepox. Monkeypox infections have occurred in species of both Cercopithecus and Colobus.

The 15 positive HI titers after KIO_4 pretreatment are somewhat difficult to interpret. If one considers HI antibody to be a reflection of recent poxvirus infection (17), there is some evidence from this study that active infections were occurring within the year preceding the surveys. Only 1 animal with HI antibodies was considered young, so we cannot conclude that these infections are usually acquired at an early age. However, the high titers of neut antibodies in several young monkeys may represent relatively recent infections. The relationship of antibody titer level and maintenance of a poxvirus infection is still unclear. A variola-like virus isolated from the kidney of a healthy chimpanzee found in the Republic of Zaire (10) was associated with a HI titer of 1:1280 and a neut titer of 1:>40 (18) indicating that premunition does occur.

It is difficult to estimate the type and intensity of contact these monkeys have with humans. In many of these areas nonhuman primates are captured and kept as pets. They are also shot for food and are often considered a culinary delicacy. Skins of Colobus monkeys are used to make cloaks and other regalia for tribal ceremonies. While nonhuman primates usually stay away from major population centers, they frequently take food from fields under cultivation.

A human monkeypox case in eastern Liberia (zone 66) (5) occurred less than 50 km. from Guiglo Department (Toulepleu region - zone 66) in the Ivory Coast where 3 of 6 Colobus badius monkeys had neut antibodies and less than 200 km. from a zone where monkeypox-specific antibody was found in the same species. A Cercopithecus mona monkey with neut antibodies was captured in Abengourou Department (Agnibilekrou region - zone 118), near the area (zone 119) where a case of human monkeypox was associated with positive neut titers in serum from Cercopithecus mona, C. diana, and C. petaurista monkeys as well as from rodents, larger mammals, and birds (6). The 2 C. petaurista monkeys with monkeypox-specific antibodies were found within 200 km. of where this human monkeypox case occurred. These forest-dwelling monkey populations may share the same arboreal habitats (19); they have been observed mixing together. Small mammals such as rodents pass under primates and, if susceptible, could conceivably become infected by this association. The potential wide host range of

monkeypox virus was demonstrated in Holland, where a strain, isolated from anteaters and monkeys, was transmitted to rabbits and suckling mice (20).

It seems obvious that a reliable serologic test that could distinguish the various poxviruses would be an important epidemiologic tool for poxvirus research. The IFA test using cross-absorbed antigen is the most promising recent development in this field.

Isolating monkeypox virus and other poxviruses from wild animals would be helpful in defining the epidemiology of these viruses. However, experience has been that a great number of animals would have to be captured in order to isolate even a few viruses. More information is needed on the possible contacts that wild animals with evidence of prior monkeypox infection have with human populations as well as on associations between animals of the same and other species.

Despite the evidence of recent and past poxvirus infections among different animal populations in this zone, there is little reason to think that the worldwide smallpox eradication program is endangered. There have been only 20 known cases of monkeypox in humans in West and Central Africa; all have occurred since 1970 (21). All but 1 of the outbreaks have been observed in forest villages of less than 300 persons. Human-to-human transmission occurs with difficulty. Only 2 secondary cases of human monkeypox among intimately exposed family contacts have

been reported. The 6% secondary attack rate for susceptible persons in contact with human monkeypox is much less than that of classic smallpox, which ranges between 35% and 45%. Even after the mass vaccination campaigns, the West and Central African population has never been much more than 80% immune to smallpox, an immunity which appears to protect also against monkeypox. As the rural forest and savanna population has regular contact with nonhuman primates as well as with other animals with poxvirus antibodies, more human cases due to monkeypox or other Orthopoxviruses would be expected if these viruses had an affinity for man. Large numbers of human cases have not been found despite intensified surveillance for human poxvirus disease since 1970.

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TABLE 1

WILD PRIMATES KILLED IN WEST AFRICA
(modified from Dorst, J., and Dandelot, P., 1969)

<u>Genus and Species</u>	<u>No. Killed</u>	<u>Common Name</u>	<u>Habitat and Habits</u>
<u>Cercopithecus:</u>			Arboreal and diurnal; all forest strata colonized except floor; groups of 40-50 composed of families of 4-5; species geographically associated; may be escorted by birds (hornbills, parrots); remain in 1 area; sedentary; eat vegetable matter supplemented by insects, birds, eggs; gestation 130 days (single birth); longevity about 20 years; predators large birds (crowned eagle), carnivores.
C. aethiops	137	Grivet or green monkey	Savanna and woodland (preforest); open country; forest gallery to sleep; bands of 6-20; mix with C. petaurista.
C. mona	37	Mona monkey	Forest and galleries; lower and middle strata.
C. petaurista	31	Lesser white-nosed monkey	Forest, fringe savanna; lower forest canopy.
C. diana	10	Diana monkey	Dense forest; upper strata.
C. nictitans	<u>5</u> 220	Greater white-nosed monkey	Forest, wooded savanna; high galleries; mix with C. mona.
<u>Colobus:</u>			Arboreal; forest; troops of up to 25; eat leaves; coat highly prized by hunters.
C. badius	15	Western red colobus	Forest; upper strata.
C. polykomos	1	Western black and white colobus monkey	High forest; fringe savanna.
C. verus	<u>1</u> 17	Olive colobus monkey	High forest; thickets and lower strata; bands of 6-20; mix with C. mona and C. petaurista; eat leaves; mother carries young in mouth.
Erythrocebus patas	5	Patas or red monkey	Savanna; open country; very terrestrial; bands of 9-30; range up to 20 sq. miles; no mixing; vegetable matter; gestation 160 days.
Papio anubis	<u>4</u>	Anubis baboon	Savanna
Total	246		

TABLE 2

VARIOLA-VACCINIA ANTIBODIES FOUND IN WILD PRIMATES

Species	HI*			Neut		
	No. Tested	No. Pos.	% Pos.	No. Tested	No. Pos.	% Pos.
<i>Cercopithecus aethiops</i>	99	4	4	101	25	25
<i>C. mona</i>	29	3	10	33	6	25
<i>C. petaurista</i>	31	5	16	31	6	19
<i>C. diana</i>	6	1	17	10	0	0
<i>C. nictitans</i>	<u>5</u>	<u>1</u>	<u>20</u>	<u>5</u>	<u>1</u>	<u>20</u>
Genus Total	170	14	8	180	38	21
<i>Colobus badius</i>	15	1	7	15	5	33
<i>C. polykomos</i>	0	-	-	1	1	100
<i>C. verus</i>	<u>1</u>	<u>0</u>	<u>0</u>	<u>1</u>	<u>0</u>	<u>0</u>
Genus Total	16	1	6	17	6	35
<i>Erythrocebus patas</i>	5	0	0	5	0	0
<i>Papio anubis</i>	<u>4</u>	<u>0</u>	<u>0</u>	<u>4</u>	<u>0</u>	<u>0</u>
	195	15	8	206	44	21

* 206 were HI positive; these 195 were tested again after being treated with KIO_4 .

Table 3

Monkeys with Positive HI Titers

<u>Species</u>	<u>Number</u>	<u>Zone</u>	<u>Age</u>	<u>Sex</u>	<u>HI</u>	<u>Neut</u>
<i>Cercopithecus aethiops</i>	334	74	A	F	40	4
" "	344	75	A	M	10	< 4
" "	346	75	A	M	20	4
<i>Cercopithecus diana</i>	503	77	A	F	10	<10
<i>Colobus badius</i>	511	77	A	F	10	<10
<i>Cercopithecus nictitans</i>	530	87	A	F	10	50
<i>Cercopithecus petaurista</i>	538	98	YA	M	40	< 4
" "	541	98	A	F	20	< 4
<i>Cercopithecus aethiops</i>	387	107	A	M	20	< 4
<i>Cercopithecus petaurista</i>	557	108	YA	F	40	< 4
" "	575	117	A	F	10	250
" "	577	117	Y	F	40	450
<i>Cercopithecus mona</i>	582	117	YA	F	20	< 4
" "	581	117	A	F	40	< 4
" "	559	118	A	F	80	100

A - Adult

Y - Youth

Table 4
 Titers on Serum Tested For
 Variola-Vaccinia Group and Monkeypox Antibodies

<u>Monkey</u>	<u>Number</u>	<u>Zone</u>	<u>HI*</u>	<u>Neut*</u>	<u>IF Titer**</u>			
					<u>After absorption with vaccinia against Vaccinia</u>	<u>After absorption with monkeypox against Monkeypox</u>	<u>After absorption with vaccinia against Vaccinia</u>	<u>After absorption with monkeypox against Monkeypox</u>
Colobus badius	492	89	< 5	2500	< 8	8	< 8	< 8
Cercopithecus petaurista	576	117	5	450	< 8	64	< 8	< 8
Cercopithecus petaurista	577	117	40	450	< 8	64	< 8	< 8

* Center for Disease Control

** Rijks Instituut voor de Volksgezondheid

Figure 1
 Geographic Distribution of Orthopoxvirus Antibodies
 in Non-Human Primates

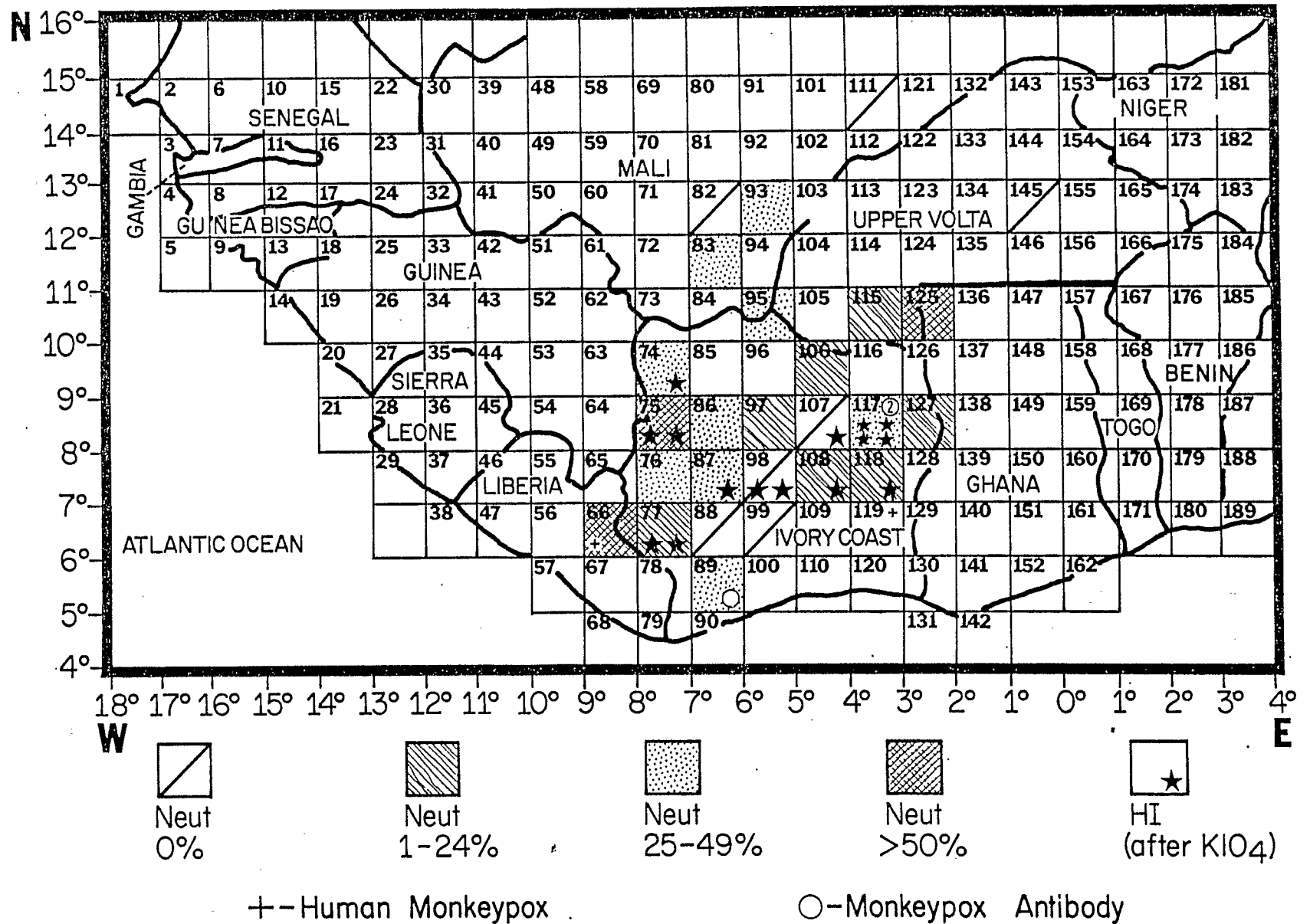


Figure 2
Neutralizing Antibody Titers in Nonhuman Primates

