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Poxvirus in West African nonhuman primates: serological survey results *

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Ten species of nonhuman primates in West African habitat were analysed for variola-vaccinia subgroup haemagglutination-inhibition (HI) and neutralization antibodies. The animals were taken in 27 different sampling zones in parts of the Ivory Coast, Mali, and Upper Volta. Of the 195 tested, 15 (8%) had elevated HI antibodies after nonspecific reactions were reduced with potassium periodate pretreatment. Positive neutralization antibodies were found in 21% (44 of 206). Antibodies were detected in serum from monkeys living near two areas where monkeypox cases in humans had occurred. Four samples were tested for monkeypox specific antibodies using an indirect immunofluorescent test; 3 were positive. Despite the 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 programme.

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 man 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 neutralization antibodies to variola-vaccinia antigen (*7*). No serum was felt to contain significant neutralization antibody. A survey of 100 Indian rhesus monkeys failed to detect either haemagglutination-inhibition (HI) or neutralization poxvirus antibodies (*8*). Other studies have shown significant variola-vaccinia group neutralization 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, surveys were carried out in areas where these primates were known to live naturally. These areas of West Africa are within the general geographical zone where human monkeypox cases occurred (*5*, *6*).

MATERIALS AND METHODS

The survey used serum samples collected in a study to define the presence of yellow fever virus circulating in monkey populations in member countries of the Organisation de Coordination et de Coopération pour la lutte contre les Grandes Endémies (OCCGE)[a] 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.

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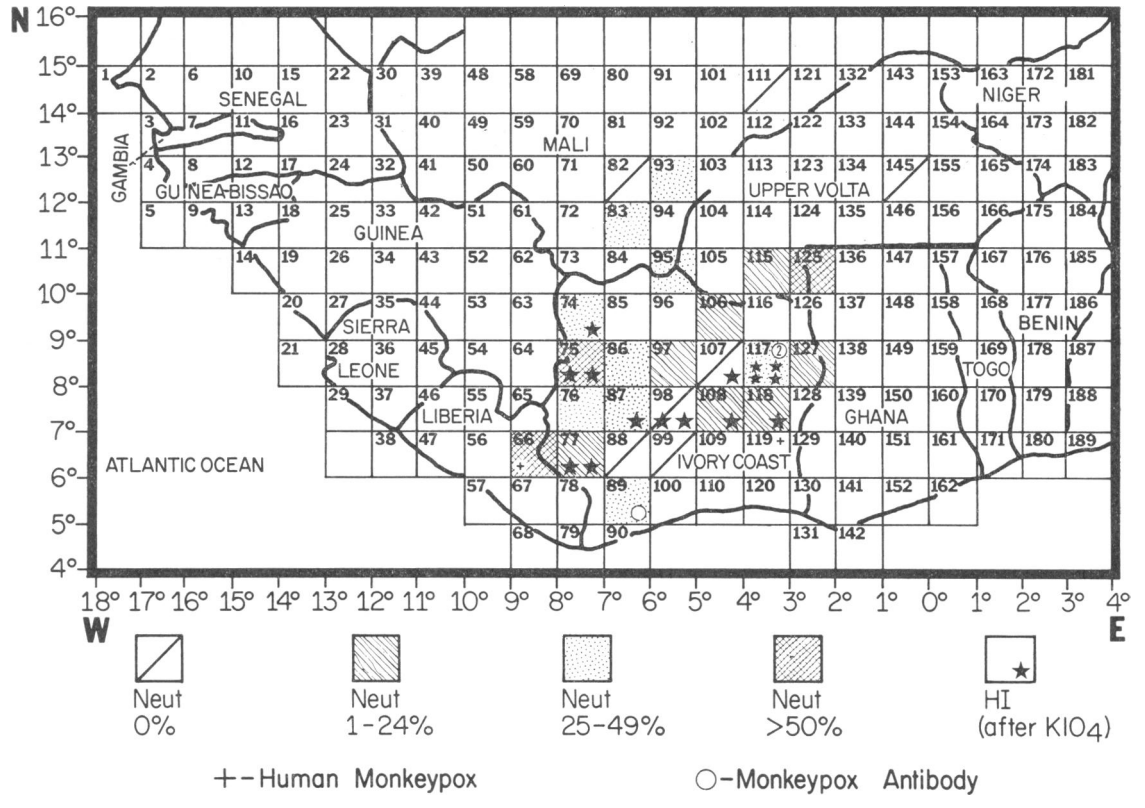

Fig. 1. Geographical distribution of orthopoxvirus antibodies in nonhuman primates.

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 bioclimatic 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 containing 4–5 drops of a solution containing penicillin, streptomycin, colistin (Colymycin), and kanamycin. Serum samples were frozen at −20°C, packed in dry ice, and sent by air to the Center for Disease Control, Atlanta, for testing.

HI antibodies to vaccinia antigen were measured at the Viral Exanthems Branch, Virology Division, Center for Disease Control, by the microtitration technique of Hierholzer & Suggs (*12*, *13*) and neutralization antibodies by a plaque reduction method (*14*). An HI antibody titre of $\geqslant$10 (reciprocal of the dilution) and a neutralization antibody titre of $\geqslant$4 were considered positive. If the HI test was positive or enough serum was available, the test was repeated after the serum had been treated with potassium periodate (KIO_4) to rule out a nonspecific reaction (*15*).

A subsample of serum that was HI- or neutralization-positive was tested for monkeypox antibody with an indirect immunofluorescence test (*16*).

RESULTS

A total of 246 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.

Sera from 206 animals were tested for HI and neutralization 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 (Table 3). Only 6 of these 15 sera had detectable neutralization antibodies. 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 neutralization antibodies. Animals with neutralization antibodies were equally divided between sexes. Among young monkeys only 11% had neutralization antibodies, whereas 27% of adults had neutralization antibodies. No sex-related differences in neutralization antibody prevalence was observed.

Fig. 2 indicates the distribution of the titres of animals with positive neutralization antibodies. *Colobus* monkeys had higher neutralization titres than *Cercopithecus* monkeys. Younger monkeys, if positive, tended to have higher neutralization titres than older specimens.

Table 1. Wild primates killed in West Africa [a]

Genus and species	No. killed	Common name	Habitat and habits
Cercopithecus			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 one 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	5	Greater white-nosed monkey	Forest, wooded savanna; high galleries; mix with *C. mona*
genus total	220		
Colobus			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	1	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
genus total	17		
Erythrocebus patas	5	Patas or red monkey	Savanna; open country; very terrestrial; bands of 9-30; range up to 50 km^2; no mixing; vegetable matter; gestation 160 days.
Papio anubis	4	Anubis baboon	Savanna
total	246		

[a] Modified from Dorst & Dandelot (*20*).

Table 2. Variola-vaccinia antibodies found in wild primates

Species	HI[a]			Neutralization		
	No. tested	No. positive	% positive	No. tested	No. positive	% positive
Cercopithecus aethiops	99	4	4	101	25	25
C. mona	29	3	10	33	6	18
C. petaurista	31	5	16	31	6	19
C. diana	6	1	17	10	0	0
C. nictitans	5	1	20	5	1	20
genus total	170	14	8	180	38	21
Colobus badius	15	1	7	15	5	33
C. polykomos	0	–	–	1	1	100
C. verus	1	0	0	1	0	0
genus total	16	1	6	17	6	35
Erythrocebus patas	5	0	0	5	0	0
Papio anubis	4	0	0	4	0	0
total	195	15	8	206	44	21

[a] 89 of 206 were HI positive; these 195 were tested again after being treated with KIO_4.

Table 3. Monkeys with positive HI titres

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

[a] A — adult, Y — young.

Table 4. Titres on serum tested for variola-vaccinia group and monkeypox antibodies

Monkey	No.	Zone	HI [b]	Neutralization [b]	IF Titre [a]			
					After absorption with vaccinia against		After absorption with monkeypox against	
					Vaccinia	Monkeypox	Vaccinia	Monkeypox
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

[a] Rijks Instituut voor de Volksgezondheid
[b] Center for Disease Control

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 had occurred.

The geographical 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.

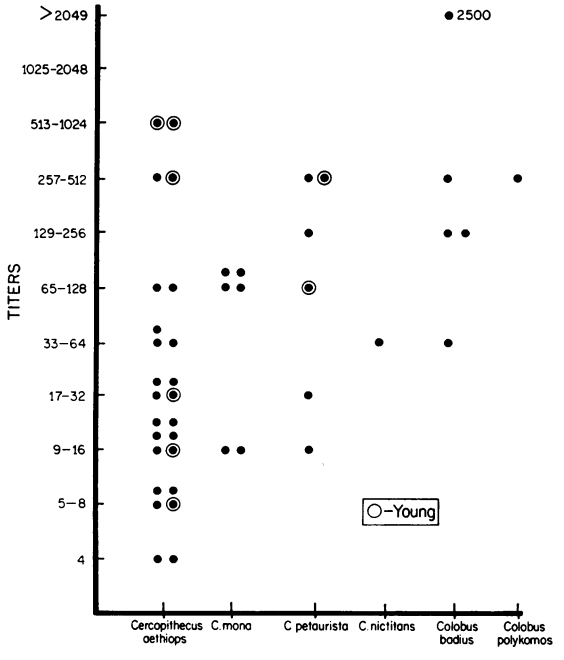

Fig. 2. Neutralization antibody titres in nonhuman primates.

DISCUSSION

These serological 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 finding that, of the HI-positive sera, only 6 had neutralization antibody is difficult to explain. It is unlikely that these other 9 monkeys had had a recent poxvirus infection but the possibility cannot be categorically excluded. Neutralization antibody was undetectable at 33 days after human smallpox infection when HI, complement fixing, and radioimmunoassay antibodies to vaccinia were present (*17*). Neutralization antibody was not observed 73 days after a primary vaccination when HI and complement fixing antibodies became elevated (Nakano, J. H., personal communication, 1977); however, these are exceptions.

It is possible that the more elevated HI titres represent relatively recent infections. HI antibody titres rise and fall more rapidly than neutralization or complement-fixing antibody titres in experimental monkeys infected with monkeypox virus (*18*). These experimental monkeys developed HI titres of between 1: 640 and 1: 1280 at 21 days which dropped to a mean of 1: 30 at 6 months. In the present study only 1 animal with HI antibodies was considered young, so we cannot conclude that these infections are usually acquired at an early age. The relationship of antibody 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 an HI titre of 1: 1280 and a neutralization titre of 1:>40 (*19*), 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 centres, 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 neutralization antibodies, and less than 200 km from a zone where monkeypox-specific antibody was found in the same species. A *Cercopithecus mona* monkey with neutralization 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 neutralization titres in serum from *Cercopithecus mona*, *C. diana*, and *C. petaurista* as well as from rodents, larger mammals, and birds (*6*). The 2 *C. petaurista* 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 (*20*); 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 the Netherlands, where a strain, isolated from anteaters and monkeys, was transmitted to rabbits and suckling mice (*21*).

It seems obvious that a reliable serological test that could distinguish the various poxviruses would be an important epidemiological tool for poxvirus research. The immunofluorescent 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 programme is endangered. There have been only 20 known cases of monkeypox in humans in West and Central Africa; all have occurred since 1970 (*22*). All but one of the outbreaks have been observed in forest villages of less than 300 persons. Person-to-person transmission occurs with difficulty. Only two 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 that appears to protect also against monkeypox. During 1975 and 1976 the health ministries of 15 countries in West and Central Africa did extensive facial pock-mark surveys in humans to detect cases of poxvirus disease that may have occurred since the last case of smallpox was confirmed in the area in 1970 (*23*). In a population of 124 600 000, a total of 6 497 250 persons were examined; 1 631 918 were preschool children, most born since 1970. None of these persons had facial pock-marks due to poxvirus.[a] It was estimated that less than 50% of the preschool children had smallpox vaccination scars. As the rural forest and savanna populations had 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.

ADDENDUM

Since this paper was prepared, it has been reported that variola, monkeypox, and vaccinia antisera can be differentiated by solid-phase radioimmunoassay after adsorption of the antisera with " homologous " and " heterologous " poxvirus-infected membranes (Hutchinson, H. D. et al., see this issue pages 613–623).

[a] *Report of the WHO International Commission for Certification of Smallpox Eradication from West Africa, 1976*, Abidjan-Brazzaville, 23 March-15 April 1976 (Unpublished WHO (AFRO) document ICP SME0 01 Afr/Smallpox/80).

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RÉSUMÉ

LES POXVIRUS CHEZ LES PRIMATES D'AFRIQUE OCCIDENTALE: RÉSULTATS D'UNE ENQUÊTE SÉROLOGIQUE

Des primates ont été capturés et saignés dans leur habitat naturel en Afrique occidentale, et la fréquence des anticorps contre les poxvirus dans leur sérum a été étudiée. Cette étude était destinée à déterminer la distribution des anticorps anti-poxvirus chez les primates dans une vaste région géographique à proximité de secteurs où des cas d'infections humaines à monkeypox s'étaient produits. On visait ainsi à évaluer la possibilité que des poxvirus trouvés chez l'animal puissent causer une maladie humaine ressemblant à la variole.

On a éprouvé 10 espèces de primates par les réactions d'inhibition de l'hémagglutination (IH) et de neutralisation afin de rechercher dans leur sérum des anticorps contre les virus du sous-groupe variole-vaccine. On a capturé 246 animaux, dont 56% étaient des *Cercopithecus aethiops* habitant la savane africaine. Ces animaux ont été pris dans 27 zones d'échantillonnage différentes en Côte d'Ivoire, au Mali et en Haute-Volta. Sur les 195 animaux éprouvés, 15 (8%) présentaient des titres élevés d'anticorps inhibant l'hémagglutination après réduction des réactions non spécifiques par prétraitement au periodate de potassium. Parmi les échantillons de sérum IH-positifs, 14 provenaient de *Cercopithecus* et 11 de singes habitant la forêt. Une proportion de 21% (44-206) des primates possédaient des anticorps neutralisants décelables. Les titres de ces anticorps étaient plus élevés chez les singes *Colobus* que chez les cercopithèques. Des anticorps ont été décelés dans le sérum de singes habitant à proximité de deux secteurs, l'un au Libéria et l'autre en Côte d'Ivoire, où des cas humains d'infection à monkeypox s'étaient produits. On a soumis 4 échantillons à une épreuve d'immunofluorescence indirecte en vue d'y rechercher les anticorps spécifiques du monkeypox; 3 de ces sérums étaient positifs et ils provenaient d'animaux capturés dans des zones situées à moins de 200 km de secteurs où des cas humains de monkeypox s'étaient produits. La plus haute concentration d'animaux positifs a été trouvée dans la savane fortement boisée et dans la forêt.

Ces enquêtes sérologiques indiquent qu'en Afrique occidentale les cercopithèques de la savane comme de la forêt et les *Colobus* de la forêt ont été infectés par un poxvirus, probablement un *Orthopoxvirus*, sous-groupe comprenant les virus de la variole, de la vaccine, du monkeypox et du whitepox. Les infections à monkeypox se sont produites chez des espèces de *Cercopithecus* et de *Colobus*. Malgré la fréquence des anticorps anti-poxvirus chez les singes et d'autres animaux en Afrique occidentale, l'éradication de la variole a été maintenue dans cette région depuis 1970. De vastes enquêtes visant à rechercher des cicatrices faciales de variole ont été effectuées en 1975 et 1976 par les ministères de la santé de quinze pays d'Afrique occidentale et centrale, mais elles n'ont pas permis de détecter des indices de variole survenue dans cette zone depuis 1970. Ainsi, les réservoirs animaux de poxvirus ne semblent pas constituer de menace pour le programme d'éradication mondiale de la variole.

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