

Outbreaks of monkeypox and serological surveys in nonhuman primates

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In connexion with the recent detection of cases of monkeypox in man in West and Central Africa, the frequency of monkeypox outbreaks in monkeys since 1958, when the disease was first recognized in captive animals, has been investigated. Special incidence surveys were made for this purpose. During the last 3 years, a serological survey has been conducted to find natural foci of monkeypox virus, and a total of 2 242 sera from monkeys of different species from various parts of Africa and Asia have been examined for poxvirus antibodies. The survey failed to detect any significant indication of poxvirus infections. The observations suggest that although a few human cases of monkeypox have been identified, monkeypox in the natural environment is not widespread and is perhaps localized in small areas.

Since 1958, when the monkeypox virus was first recognized at the Statens Seruminstitut, Copenhagen, Denmark, 9 monkeypox outbreaks have been recorded in captive monkey colonies in laboratories or zoos (Arita & Henderson, 1968) and a tenth has been recently reported from the Centre d'Enseignement et de Recherches de Médecine aéronautique, Paris (C. Milhaud, Klein & Virat, 1969). However, no naturally occurring monkeypox in wild monkey populations has been reported during the last two decades. Furthermore, in the 10 recognized outbreaks in captive monkey colonies, no human infection with monkeypox was observed despite the close contact of animal handlers with the infected monkeys.

Quite unexpectedly, however, between August 1970 and April 1971, 7 cases of human infection with a monkeypox-like virus were discovered in remote rural areas of Sierra Leone, Liberia, the Democratic

Republic of the Congo,⁸ and Eastern Nigeria (*Wkly epidem. Rec.*, 1971, and B. Lourie, personal communication). Epidemiological evidence suggests that the virus was transmitted from wild monkeys, or possibly other mammals, to individual patients through unusually close contact. In these areas, monkeys are frequently eaten and their skins processed. However, despite the fact that a number of susceptible persons came into contact with the monkeypox patients, no secondary cases occurred. The failure of man-to-man transmission may have been a chance occurrence resulting from inadequately close contact or inadequate excretion of virus by the patients, or both; or it may indicate that monkeypox virus is not transmitted man-to-man.

Since 1967, the World Health Organization and its collaborating laboratories have undertaken a number of cooperative studies to determine the extent and natural behaviour of poxvirus infections in monkeys. Efforts have been made to identify all known outbreaks of monkeypox, and natural foci of this virus have been sought by means of serological surveys. The results of these surveys are presented in this report.

MONKEYPOX OUTBREAKS IN CAPTIVE MONKEYS

In 1968 and again in 1970, laboratories and other biological centres that handle large numbers of non-

⁸ Renamed the Republic of Zaire on 27 October 1971.

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keys were asked by WHO whether they had observed outbreaks of monkeypox, and if so to provide detailed information on such outbreaks and to ascertain whether human infections had occurred. Information was obtained from 27 institutions in 11 countries in 1968, and from 51 institutions in 25 countries in 1970 (Table 1). Epidemiological details of 9 outbreaks that were recognized at the time of the first

survey were reviewed by Arita & Henderson (1968). Only one additional outbreak has been recognized since. This outbreak occurred in France in November 1968, at which time one of two chimpanzees imported from Sierra Leone developed an infection clinically resembling monkeypox. Virological studies conducted at that time suggested that the causative agent resembled monkeypox virus and this was confirmed by further studies made in 1971 (R. Netter, personal communication).

Table 1. Monkeypox outbreaks in captive monkeys, and number and location of laboratories surveyed

Country	No. and year of monkeypox outbreaks in captive monkeys	No. of laboratories that replied but reported no outbreaks	
		1st survey (1968)	2nd survey (1970)
Cambodia	—	—	1
Cameroon	—	—	1
Canada	—	1	1
Czechoslovakia	—	1	4
Denmark	1 (1958)	1	1
France	1 (1968)	2	3
Greece	—	—	1
Guadeloupe	—	—	1
Guinea	—	—	1
Hungary	—	1	1
Iran	—	—	1
Italy	—	1	1
Japan	—	1	1
Madagascar	—	—	1
Morocco	—	—	1
Netherlands	2 (1964–65)	3	2
New Caledonia	—	—	1
Senegal	—	—	1
South Africa	—	—	1
Sweden	—	1	1
Tunisia	—	—	1
USSR	—	4	1
UK	—	—	3
USA	6 (1959–66)	11	18
Yugoslavia	—	—	2
total	10	27	51

In all, 10 outbreaks have been recognized: 1 in Denmark, 1 in France, 2 in the Netherlands, and 6 in the USA; all occurred during 1958–68. Seven of the outbreaks were reported in the literature; the three others came to light as a result of the special enquiries. No human infections with monkeypox virus were observed in any of the outbreaks.

INITIAL SEROLOGICAL SURVEY

During the period 1967–69, several biological laboratories¹ provided sera from monkeys obtained in South-East Asia and Africa. As shown in Table 2, 1 614 sera from 14 species of monkey were collected. The sera were tested for poxvirus antibody in six laboratories in France, Japan, the Netherlands, the USSR, and the USA.² Three laboratories tested all sera for neutralizing antibody and three screened the sera first for haemagglutinin-inhibiting (HI) antibody and measured neutralizing antibody only when HI antibody was present.

A total of 1 114 sera were examined for neutralizing antibody; only 2 showed antibody and these were weakly positive at dilutions of 1 : 8 and 1 : 16, respectively. These two were among a group of sera from *Macaca irus* monkeys (source unknown). However, on account of the small quantities of sera available, no further tests were carried out.

The HI test showed nonspecific inhibition in some of the sera, but when these were further tested for neutralizing antibody, significant levels of antibody could not be detected.

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Table 2. Distribution of monkey sera by species of monkey and area of capture

Species	No. of sera	Area of capture	Year of bleeding
<i>Macaca fuscata</i>	64	Japan	1967
<i>M. irus</i>	378	Philippines, Indonesia, Malaysia, Thailand	1965-69
<i>M. mulatta</i>	93	India	1967
<i>M. radiata</i>	72	India	1967
<i>M. cyclopis</i>	20	unknown	1963-68
<i>M. speciosa</i>	9	unknown	1963-68
<i>Cercopithecus aethiops</i>	170	Chad, Upper Volta, Mali, Kenya, Senegal	1967-68
<i>Erythrocebus patas</i>	265		
<i>Papio</i> sp.	268		
gorilla	25	unknown	1963-68
chimpanzee	167		
orangutan	40		
gibbon	8		
marmoset	35		
total	1 614		

SPECIAL SEROLOGICAL SURVEY OF *MACACA IRUS* MONKEYS IN MALAYSIA

At least four of the virologically confirmed monkeypox outbreaks in captive monkey colonies during 1958-66 occurred among *Macaca* monkeys from the Malaysian Peninsula, shipped from Kuala Lumpur (Table 3). It was decided, therefore, that a special serological survey should be undertaken of *M. irus* monkeys captured in that area.

Sera were obtained through the cooperation of the Institute for Medical Research, Kuala Lumpur. Along the western coast and in the inland area from Perlis District to Johore District, 23 trapping areas were established. From September 1969 to January 1970, 481 *M. irus* monkeys were captured. Some 50% of the monkeys were under 4 years of age, 49% were between 5 and 9 years, and 1% were over 10 years old; males and females were almost equally represented. Captured monkeys were transported to Kuala Lumpur where they were bled. Of 481 sera obtained, 70% were collected within 4 days after the capture of the animal, and the remaining sera were obtained within 9 days, depending upon the distance from the trapping areas to the bleeding site. All the

monkeys were in good health and no evidence of pox infection was observed. Sera were distributed to four participating laboratories¹ for testing.

For screening purposes, qualitative tests for pox-virus neutralizing antibodies were carried out by the individual laboratories. Details of the test methods are given in Table 4. Laboratories A, C, and D employed neutralization tests with pock count on chorioallantoic membrane at a serum dilution of 1:4 and laboratory B conducted neutralization tests with 50% plaque reduction in tissue-culture at dilutions of 1:5 and 1:15. Monkeypox virus antigen was used for neutralization tests by laboratories A and D, and vaccinia virus by laboratories B and C. In addition, laboratory A determined antibody by indirect immunofluorescence and laboratories B and D also determined the level of HI antibody.

The results of tests in the four laboratories are summarized in Table 5. In laboratory A, all the 154 sera tested for neutralization and immuno-

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Table 3. Origin of monkeys that caused outbreaks of monkeypox in institutions

Outbreak	Place of outbreak	Year	Monkeypox virus isolated from:	Shipped from:	Reference
1	Statens Serum Institute, Copenhagen, Denmark	1958	<i>Macaca philippinensis</i>	Malaysia	Magnus et al. (1959)
2	Rotterdam Zoo, Netherlands	1964-65	giant anteater and orangutan	Close contact with monkey from Malaysia	Gispén, Verlinde & Zwart (1967)
3	National Institute of Public Health, Utrecht, Netherlands	1964-65	healthy monkey kidney tissue-culture	Malaysia	Gispén, Verlinde & Zwart (1967)
4	Walter Reed Army Institute, Washington, D.C., USA	1961	<i>M. irus</i>	?	McConnell et al. (1964)
5	Merck, Sharp & Dohme, Research Laboratories, West Point, Penn., USA	1959	<i>M. philippinensis</i>	Malaysia	Prier et al. (1960)
6	National Center for Primate Biology, Davis, Calif., USA	1966	langur	?	C. Espana, personal communication
7	Lederle Laboratories, Pearl River, New York, N.Y., USA	before 1966	<i>M. mulatta</i> ^a	India	J. H. Vickers, personal communication, 1967
8	The Dow Chemical Company Biological Laboratories, Zionsville, Ind., USA	1965	<i>M. irus</i> ^a	Philippines or Malaysia	A. H. Bruschner, personal communication, 1967
9	Wyeth Laboratories, Inc., Marietta, Penn., USA	1966	<i>M. mulatta</i> ^a	India	M. Z. Bierly, personal communication, 1967
10	Centre d'enseignement et de Recherches de Médecine Aéronautique, Paris, France	1968	chimpanzee	Sierra Leone	Milhaud, Klein & Virat (1969)

^a Virus isolation was not carried out.

fluorescence reactions showed negative results, except one that repeatedly showed a titre of 1 : 8 by immunofluorescence but contained no detectable neutralizing antibody or HI antibody. The significance of this observation is uncertain.

Laboratory B tested 94 sera with plaque-reduction tests for neutralizing antibodies. Altogether, 8 sera showed neutralizing antibody at a 1 : 5 dilution but all were negative at a dilution of 1 : 15; 11 of 94 sera showed HI inhibiting antibody at a dilution of 1 : 5 but were negative at a dilution of 1 : 10; 4 sera contained HI antibodies at low titre but no neutralizing antibodies, and 1 contained neutralizing antibody at low titre but no HI antibody. It is possible that some or all of these positive reactions may have been nonspecific.

Of 96 sera tested by laboratory C for the presence of neutralizing antibody at a dilution of 1 : 4, 14 sera showed a reduction of 50-75% of the number of pocks at a 1 : 4 dilution; this response was considered to be nonspecific.

None of the 137 sera tested by laboratory D were found to contain either neutralizing antibodies or HI antibodies at a dilution of 1 : 4.

SPECIAL SEROLOGICAL SURVEY OF MONKEY SERA FROM CHAD, WEST AFRICA

In June 1970, sera of 147 patas monkeys from Chad were collected through the Institut Merieux, France. The monkeys were bled in that institute 1½-3 months after capture. Laboratory D con-

Table 4. Neutralization tests employed to screen monkey sera in laboratories

Laboratory	Screening method	Strains of challenge virus	Diluent for virus	Hours of contact between virus and serum at 37°C	Dilution of serum	No. of eggs or tubes inoculated with each dilution	Pre-treatment of serum
A	Pocks on chorioallantoic membrane 72 hours after inoculation	monkeypox virus (Copenhagen strain)	10 % skimmed milk in distilled water	2	1 : 4	at least 6	56°C for 30 minutes
B	Plaques in tissue-culture of monkey kidney (50 % reduction)	vaccinia (IHD)	phosphate-buffered saline	2	1 : 5 1 : 15	2	not inactivated
C	Pocks on chorioallantoic membrane 48 hours after inoculation	vaccinia (EM-63)	McIlvaine buffer solution (0.004M, pH 7.2)	2	1 : 4	5	56°C for 30 minutes
D	Pocks on chorioallantoic membrane 72 hours after inoculation	monkeypox virus (Copenhagen strain)	0.2 % gelatin-phosphate-buffered saline	1 ^a	1 : 4	4	56°C for 30 minutes

^a In addition, the mixture was subsequently allowed to stand overnight at 4°C.

ducted neutralization tests and HI tests. In the neutralization test, 17 sera showed weakly positive responses (50–70% pock reduction) at a serum dilution of 1 : 4, but on repeat testing at dilutions of 1 : 4, 1 : 8, and 1 : 10 all sera were negative. The remaining 130 sera were negative at a dilution of 1 : 4. None of the 147 sera showed HI antibody at a dilution of 1 : 4 except one that was subsequently found to have no neutralizing antibody.

In summary, a total of 2 242 monkey sera (1 614 from the initial survey, 481 from the Malaysia survey,

and 147 from the Chad survey) were tested, but none had significant levels of neutralizing antibody.

DISCUSSION

Since 1958, when an outbreak of monkeypox was first recognized in the Statens Serum Institut, Copenhagen, only 10 outbreaks among captive monkeys have been recorded. Arita & Henderson (1968) reported that the clinical manifestation of the disease is distinctive and the disease would be readily

Table 5. Results of serological tests of *M. irus* monkey sera from the Malay Peninsula

Laboratory	No. of sera tested	Neutralization test	HI test	Immuno-fluorescence test
A	154	all negative at 1 : 4 dilution	— ^a	1 serum positive at 1 : 8 dilution
B	94	8 sera positive at 1 : 5 dilution but all negative at 1 : 15 dilution	11 sera positive at 1 : 5 dilution but all negative 1 : 10 dilution	— ^a
C	96	14 sera weakly positive at 1 : 4 or lower dilution	— ^a	— ^a
D	137	all negative at 1 : 4 dilution	all negative at 1 : 4 dilution	— ^a
total	481			

^a Not done.

suspected by laboratory workers who have become increasingly cautious in recent years with regard to the health of animals newly arrived in laboratories. Approximately 130 000 primates were imported into the USA in 1968, and during the last 3 years the number of monkeys imported was probably about 400 000. Despite the large number of monkeys involved, none of the laboratories in the USA recorded outbreaks of monkeypox during this period. It is not possible to estimate how many primates are imported into Europe. However, considering the increasingly frequent use of monkeys by laboratories in America and Europe, 10 monkeypox outbreaks during the last 12 years appears to be extremely few.

The serological surveys aimed at defining the natural foci of monkeypox failed to detect any significant level of poxvirus antibodies in a total of 2 242 monkey sera originating from South-East Asia and the African continent south of the Sahara. Moreover, 156 sera from Chad and Senegal tested for HI antibodies by the Center for Disease Control, Atlanta, USA, gave negative results for poxvirus (Noble, 1970). In the initial survey described in this report, the monkeys were not bled until long after their capture, and there could have been ample opportunity for exposure to monkeypox infection, during transportation and while they were kept in colonies with other animals. These monkeys can therefore be regarded as a sensitive indicator for the presence of monkeypox infection; the evidence is that there was no such infection.

Of the 10 known outbreaks, 4 occurred in *Macaca* monkeys that came from Malaysia. However, a special serological survey of the same species of monkey in Malaysia again failed to detect monkeypox infection in that area. The sera were taken in 1969, 4 years after the last known monkeypox outbreak (1965) originated in monkeys from this area, but half of the animals from which the sera were obtained were born before this outbreak. Gispén (personal communication) showed that monkeypox neutraliz-

ing antibody persists for long periods after infection. He found a high titre of neutralizing antibody (1:160 and 1:700) in sera from two orangutans naturally infected with monkeypox virus in the Rotterdam Zoo 6 years previously. It seems reasonable to assume, therefore, that if any of the monkeys had been infected in the past, neutralizing antibody would have been detected.

Hahon (1961) observed the initial symptom of rise in temperature in *M. irus* monkeys 4 days after aerosol infection with monkeypox virus. Assuming that the average number of days between the date of exposure and occurrence of infectivity caused by excretion of virus is 1 week, a minimum of 52 monkeys a year should be infected, or produce significant antibody titres when an infected animal is introduced into a monkey population, if the disease is to be sustained in a given area. Apparently, however, so tenuous a line of transmission tends to cease and much greater numbers of susceptible monkeys are required to sustain the infection over a long period. Furthermore, if continuous transmission of monkeypox in monkeys followed the pattern of smallpox in human populations, transmission would continue until most susceptible monkeys had been infected, as observed in the variola minor outbreak in a Brazilian village in 1970 (N. Arnt & L. Morris, personal communication). These considerations would suggest that if monkeypox were being maintained in wild monkey populations, the negative results in all monkey sera tested is an extremely unusual phenomenon.

At present, it is difficult to understand the natural ecology of monkeypox. However, it can be stated that monkeypox virus is not widespread in the natural environment and is perhaps localized in limited areas. Further studies are certainly warranted, particularly in the areas where human monkeypox cases have occurred sporadically. Particular attention might be given to an area of Sierra Leone whence the infected chimpanzee originated in 1968 and where a human case was recognized in 1970.

RÉSUMÉ

ÉPIZOOTIES DE MONKEYPOX ET ENQUÊTES SÉROLOGIQUES CHEZ DES PRIMATES NON HUMAINS

Depuis 1958, date de la première identification de la maladie au Statens Serum Institut de Copenhague, on n'a signalé qu'une dizaine d'épizooties de monkeypox chez des singes en captivité. Ce chiffre est très bas, étant donné le

nombre considérable de primates importés durant la même période en Europe et en Amérique.

En 1967/69, lors d'une première enquête sérologique visant à découvrir des foyers naturels de monkeypox,

on n'a pu déceler de titres appréciables d'anticorps anti-poxvirus dans un total de 1614 sérums prélevés chez des singes capturés en Asie du sud-est et en Afrique au sud du Sahara. En raison du long intervalle entre la capture et le prélèvement des sérums, ces animaux ont eu de multiples occasions de contracter le monkeypox au cours du transport ou au contact de leurs congénères déjà en colonies, mais on n'a constaté aucun signe d'une telle affection.

Quatre au moins des poussées de monkeypox étant survenues chez des singes *Macaca irus* originaires de Malaisie, on a examiné en 1969/70 les sérums prélevés chez 481 singes de cette espèce capturés dans les environs de Kuala Lumpur. Ici encore, les épreuves de neutrali-

sation, d'inhibition de l'hémagglutination d'immuno-fluorescence n'ont apporté aucune preuve de la présence du virus du monkeypox dans cette région.

En juin 1970, les sérums de 147 singes patas originaires du Tchad ont été examinés sans succès en épreuves de neutralisation et d'inhibition de l'hémagglutination.

A l'heure actuelle, les caractéristiques écologiques du virus du monkeypox sont peu connues. On peut cependant affirmer que le virus n'est pas très répandu dans la nature et qu'il ne circule peut-être que dans certains foyers localisés. Il conviendrait de poursuivre les recherches, notamment dans les régions où des cas humains sporadiques ont été signalés.

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