The African Journal of MEDICAL SCIENCES

Volume 3

1972

BLACKWELL SCIENTIFIC PUBLICATIONS
Oxford London Edinburgh Melbourne

Afr. J. med. Sci. (1972) **3**, 177–185.

Isolation of *Herpesvirus hominis* from Lemurs: A Naturally Occurring Epizootic at a Zoological Garden in Nigeria

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(*Received* 28 *June* 1971)

Summary. During the period January–August 1968, a fatal illness occurred in a small colony of lemurs (*Lemur catta*) established at the University of Ibadan Zoological Garden in late 1962. *Herpesvirus hominis* was isolated from the brain and saliva of one lemur and from the brain of another. Histopathologic examination showed lesions compatible with herpesvirus infection in tissues of lemurs and in mice inoculated with lemur material. Identity of the virus was established by neutralization tests in mice and tissue culture systems utilizing human foetal kidney diploid cells and primary rhesus monkey kidney cells in conjunction with known *Herpesvirus hominis* hyperimmune serum; and in fluorescent antibody tests done with infected mouse brain material and specific hyperimmune sera conjugated to fluorescein-isothiocyanate.

Résumé. Entre Janvier et Août 1968 un maladie mortelle s'est répandue dans une petite colonie de lemurs (*Lemur catta*) établie au jardin zoologique de l'Université d'Ibadan vers la fin de 1962. *Herpes hominis* a été isolé de la cervelle et de la salive d'un lemur et de la cervelle d'un second. L'examen histopathologique a montré, dans les tissus des lemurs et des souris inoculées avec du material provenant de lemurs, des lesions qui ressemblent à celles de l'infection avec le virus de l'herpes. L'identification du virus a été faite par les tests de neutralisation chez la souris et en culture sur des cellules diploides de rain fetal humain et les cultures primaire de cellules de singe rhesus avec du serum hyperimmune specific pour *Herpes hominis*; et par les anticorps fluorescents sur des preparations de cervelle de souris infectées, avec le serum hyperimmune conjugé à la fluorescein isothiocyanate.

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INTRODUCTION

Man historically has had the desire to harbour animals for beauty, utility and amusement More recently man has made increasing use of subhuman primates and other exotic animals for purposes of medical research and the production of beneficial biologic products. Transmission of disease from animals to man is well known. Less frequently reported are those occasions when man infects the animals under his care with his own parasites. As animals are maintained in increasingly large numbers under relatively crowded conditions, the potential for epizootic disease amongst them increases. A number of recent reports suggest that natural, fatal infections of subhuman primates and other vertebrates with *Herpesvirus hominis* may be more common than previously supposed (Emmons & Lennette, 1968, 1970; Smith *et al.*, 1969; Melendez *et al.*, 1969; Hunt & Melendez, 1969).

This paper reports a fatal illness in a small colony of ringtailed lemurs (*Lemur catta*) at the University of Ibadan Zoological Garden, and the isolation of *Herpesvirus hominis* from the animals. Lemurs as a genus have not hitherto been known to be susceptible to infection with this virus.

MATERIALS AND METHODS

Laboratory techniques used at the Ibadan Laboratory and at the Viral and Rickettsial Disease Laboratory, California State Department of Public Health, Berkeley, U.S.A., have been reported elsewhere (Causey *et al.*, 1970; Lennette & Schmidt, 1969) and are only briefly related here.

Blood samples were allowed to stand at room temperature until the clot had retracted, usually within 1–2 hr, and then were centrifuged to obtain serum. Tissues were triturated as a 20% suspension in 0·75% bovine plasma albumin (fraction V) in phosphate-buffered saline containing 2000 units of penicillin and 0·6 mg of streptomycin/ml. Following centrifugation at 3000 rpm for 5 min, the supernatant fluid was used as inoculum. Saliva samples were prepared as a 10% solution with bovine albumin as described for tissues. All samples were inoculated intracerebrally (ic) into 1- or 2-day-old mice (albino Swiss mice, Webster strain, originally obtained from The Rockefeller Foundation), and the mice were observed for 14 days for signs of illness.

Tests of virus isolates for filtrability and sensitivity to chloroform or ether were conducted when indicated. Identification of virus was made by complement-fixation (CF) and neturalization (N) tests with a hyperimmune mouse ascitic fluid (MAF) for *Herpesvirus hominis*, strain HF, supplied by the Yale Arbovirus Research Unit, New Haven, Connecticut, or with hyperimmune rabbit serum for *Herpesvirus hominis*, MacIntyre strain. Passage attempts and virus titrations in suckling (2–3 days old) and adult mice and in human foetal kidney diploid (HFDK) cell cultures and primary rhesus monkey kidney (MK) cell cultures were performed according to standard methods. For N tests in suckling mice, the constant serum, varying decimal dilution of virus method was used. Immune sera were inactivated at 56°C for 30 min, and serum–virus mixtures were held at 37°C for 1 hr before inoculation ic. LD_{50} titres were calculated by the method of Reed & Muench (1938). For N tests in HFDK cell culture systems, also done with heat-inactivated serum, 10 and 100 infectious doses ($TCID_{50}$) of virus were incubated for 1 hr at 37°C with two-fold dilutions of serum; after inoculation of the serum–virus mixtures, the cell cultures were observed for 10 days.

Fluorescent antibody (FA) staining methods were as described for rabies FA staining by

Lennette *et al.* (1965). In the direct method, specific hyperimmune sera conjugated to fluorescein-isothiocyanate were used to stain infected mouse brain or cell cultures.

Lemur tissues were examined grossly for lesions during autopsy, and brain, liver, kidney, lung, spleen, heart and small and large intestine were placed in 10% formalin. Suckling mice that became obviously ill after inoculation with lemur material were sacrificed, the cranium, abdomen and thorax opened and the entire carcass fixed in Bouin's fluid; seven transverse sections of the body were made at approximately equal intervals from head to pelvis. Standard paraffin sections of both lemur and mouse tissues were cut at 4–6 μm (n) and stained with haematoxylin and eosin.

DESCRIPTION OF THE EPIZOOTIC

The lemur colony was established at the Zoological Garden in late 1962 with four animals, and during succeeding years a number of lemurs were born and reared at the Zoo. Until December 1967 the colony was housed in cage F (see Fig. 1) in the general primate section, within arm's-length reach of an adjacent cage containing six *Cercopithecus mona* and two *C. aethiops*. Cage F is separated from the public by a high hedge. As part of a modernizing

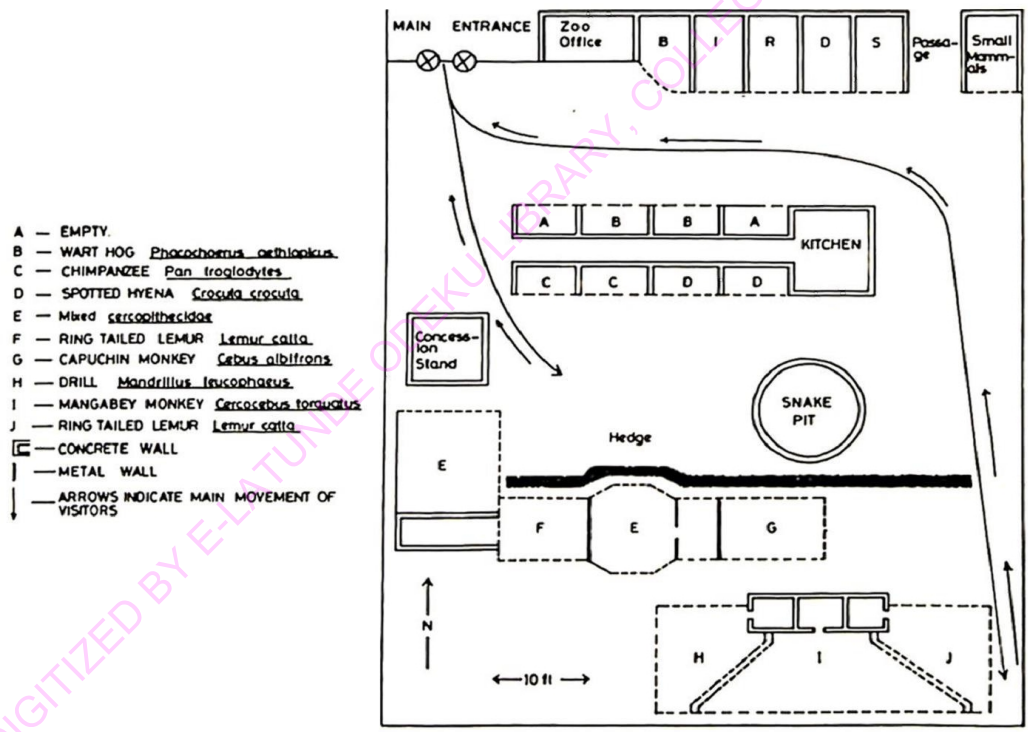

FIG. 1. Diagram of a portion of university of Ibadan Zoological Garden.

programme at the Zoo, a new cage (J) was built to show the lemurs to better advantage, and to this new cage, never before occupied, the colony was moved on 12 December 1967. Cage J is situated about 30 m from cage F and has direct exposure to the public since it is on the main thoroughfare through the Zoo. A special feature of its construction was a

TABLE 1. Case histories and laboratory data on lemurs

Lemur no.	Sex birth date	Illness (1968) Onset	Illness (1968) Death	Gross pathology	Histopathology	Herpesvirus isolation history		Remarks
1	Male	20/1	25/1	Mild keratitis	Extensive encephalitis, meningitis, no inclusions	Brain	Yes	On 20/1 listless sitting with odd posture. Thought to have middle ear infection
						Liver	No	
						Spleen	No	
2	Female 3/67	5/2	6/2	Hindquarters soiled with faeces. Mucosa of small intestine hyperemic	Localized encephalitis; eosinophilic intranuclear inclusions	Blood (5/2)	No	Salivating heavily on 5/2. Suspected to have been poisoned
						Faeces (5/2)	No	
						Saliva (5/2)	Yes	
						Blood (6/2)	No	
						Brain (6/2)	Yes	
						Liver (6/2)	No	
						Lung (6/2)		
						Spleen (6/2)		
						Kidney (6/2)		
3	Female 1962	11/2	21/8		Autopsy not done	Saliva 15/2	No	Salivating heavily on 11/2. Aborted foetus (Lemur 4) on 15/2; carrying head to one side. In April, moving slowly and only able to jump short distances
4	? 15/2/68	—	15/2		Autopsy not done	Umbilicus	No	Foetus aborted by Lemur 3
						Brain	No	
						Liver	No	
						Spleen	No	
						Stomach	No	
						Lung and heart	No	
						Kidney	No	

Table 1 (*cont.*)

Lemur no.	Sex birth date	Illness 1968 Onset	Illness 1968 Death	Gross pathology	Histopathology	Herpesvirus isolation history		Remarks
5	Male 4/64	5/2	11/6	No visible lesions	Hypertrophy and hyperplasia of bile duct epithelium	Not examined		About 5/1 lethargic with unsteady gait. On 13/2 appeared normal. On 27/3 found lethargic on ground. In May still weak and unable to jump
6	Female 3/63	29/3	26/5		Autopsy not done	Not examined		Mother of Lemur 2. On 15/2 given human-origin gamma-globulin. On 29/2 gave birth to Lemur 7; became aggressive. On 29/3, lethargic. On 6/5, very listless
7	? 29/2/68				Disappeared 16/5	Not examined		Progeny of Lemur 6. Small at birth, but normal. Developed slowly but appeared healthy
8	Female 3/65	26/3	27/3	No visible lesions	No abnormal findings	Saliva 2/3	No	On 2/3 given gamma-globulin. On 4/3 thought to have aborted. On 26/3 extremely lethargic lying on ground

partially enclosed portion in which pregnant lemurs or lemurs with their young could be separated from the main cage and gain better protection from the elements.

In cage J, the lemurs were 10–15 ft distant from cages containing *Mandrillus leucophaeus* and *Cebus albifrons*, and theoretically, at least, had no direct contact even with *Cercocebus torquatus* in the adjacent cage, although they could have touched hands with the last by reaching around the end of a solid wall.

Illness in a lemur was first noted in January 1968; case history data are given in Table 1. On 20 January, Lemur 1, a male born prior to 1962, was observed to be listless and sitting with an 'odd posture.' He was thought to have a middle ear infection and was treated with antimicrobials on the third and fourth days of his illness. On 25 January, Lemur 1 died.

Eleven days later (5 February) Lemur 2, a female born at the Zoo in 1967, was noticed to be salivating heavily and showing extreme weakness. Poisoning was suspected. Lemur 2 died on 6 February.

In an attempt to prevent further losses, the remaining lemurs were moved back to cage F on 11 February. Following this move, Lemur 3, an adult female, was noted to be salivating copiously. On 15 February an aborted foetus (Lemur 4) was found in the cage with her. At this time Lemur 3 was also observed to be carrying her head to one side. As lemurs are considered valuable zoological specimens, she was given 0·5 ml of human gamma-globulin intramuscularly on the 15 February and, by way of separating her from Lemur 6 who was thought to be pregnant, was isolated in new cage J. In April, Lemur 3 was still moving slowly and with great care; she would jump only a very short distance. On 8 May she was still dull and appeared thin. Lemur 3 was found dead on 21 August.

Lemur 5, a male born at the Zoo in 1964, seemed lethargic and unsteady at the time of Lemur 2's illness and death (5–6 February), but showed no excess salivation. Within 2 days of the return to cage F on 11 February, his behaviour appeared to be normal. On 27 March, however, he was found lying on the ground, and his movements were lethargic. The next day he was put back in cage J with Lemur 3. Lemur 5 now held his head to one side, and his equilibrium was noticeably affected. In May he was still weak and unable to jump. He died on 11 June.

As already mentioned, Lemur 6, born at the Zoo in 1963, was believed to be pregnant in February 1968. On the 15th of that month, along with Lemur 3, she was given human gamma-globulin, 0·9 ml intramuscularly. On 29 February she gave birth to a normal offspring (Lemur 7). At this time she became aggressive, but by 29 March her actions were lethargic. On 6 May she was noticed to be very listless. Lemur 6 died on 26 May.

Lemur 7, the baby of Lemur 6, was small at birth and developed slowly, but seemed well at all times. On 18 March it began to climb on the wire and on 23 March moved to its mother's back (a normal pattern of development in this species). During the night of 16 May, Lemur 7 disappeared.

At the beginning of the preceding March, Lemur 8, born at the Zoo in 1965, was thought to be pregnant and on 2 March she was given 0·5 ml of gamma-globulin; Lemur 6 apparently having benefited from her injection. The next day, Lemur 8 appeared normal, but on 4 March her abdomen was less distended and she was assumed to have aborted. On 26 March she became extremely lethargic, lying on the ground a great deal. On the 27th she was very weak, and died that day.

RESULTS

(*A*) *Virus isolation*

Of the five lemurs tested for virus, isolations were made only from Lemurs 1 and 2 (Table 1).

Lemur 1. Mice groups inoculated with liver and spleen suspensions remained well through 14 days, but in the group inoculated with brain suspension (IbAn 24400) one mouse died on day 4 post-inoculation (PI) and by day 5 PI the others had died or were ill. Smears made from the brain of one of these mice was checked for bacterial contamination, with negative results.

Most of the 12 second-passage mice were ill on day 2 PI. No bacteria were detected when impression smears prepared from the surface of the brain of one of these mice was stained with Seller's stain and examined by light microscopy.

IbAn 24400 virus was reisolated from a portion of the original material stored at −60°C for about 9 months.

A *Herpesvirus hominis* (strain HF) MAF had a neutralizing index (NI) of 2·2 dex (Haldane, 1960) against the virus in N test.

Additional studies at the Viral and Rickettsial Disease Laboratory, California State Department of Health, confirmed the identity of IbAn 24400 virus as *Herpesvirus hominis*. The virus titre by ic inoculation of suckling mice was 6·9 dex/ml, and the mice died in 3–5 days; the titre in 3- to 4-week-old mice was 7·7 dex/ml, and the mice died in 4–6 days. In MK cell cultures inoculated with infected mouse brain (mouse passage 3, HFDK passage 2), no cytopathic effect (CPE) was visible through day 10 PI. In contrast, HFDK cell cultures inoculated with mouse brain stock virus showed *Herpesvirus*-type CPE (graded 3+) with rounded, refractile cells by day 7 on initial passage and by day 3 on subsequent passages. The virus titre in HFDK cells was 6 dex/ml, and CPE was completely inhibited through day 10 (10 and 100 $TCID_{50}$) by *Herpesvirus hominis* (MacIntyre) rabbit serum to a titre of 1 : 32. The homologous titre of the serum, determined simultaneously, was 1 : 64.

FA staining of the infected mouse brains and HFDK cell cultures was unequivocally positive for *Herpesvirus hominis*, while FA tests for rabies, vaccinia and varicella viruses were negative.

Lemur 2. Of the three specimens secured on 5 February, virus was isolated only from saliva (IbAn 24664). This isolate reacted in CF test with a *Herpesvirus hominis* MAF. Re-isolation was not attempted.

Of the tissues collected from Lemur 2 at autopsy on 6 February, only brain material (IbAn 24668) yielded virus. A *Herpesvirus hominis* MAF had a NI of 1·9 dex against this isolate in N test.

(B) *Pathological and histopathological findings in lemurs*

The findings on gross examination are summarized in Table 1.

On histopathological examination, Lemur 1 showed evidence of a fairly extensive diffuse encephalitis characterized by neuronal necrosis and glial nodules. Perivascular infiltration by mononuclear cells was common. The endothelial cells were hyperplastic and hypertrophied. Inclusion bodies were not observed. There was a meningitis characterized by mononuclear infiltration. There were no visible lesions in other tissues examined.

Lemur 2 had a localized encephalitis in the diencephalon characterized by perivascular

cuffing with mononuclear cells, swelling and proliferation of endothelial cells, and the presence of glial nodules near blood vessels. In areas of malacia, there were numerous glial astrocytes with well-defined eosinophilic intranuclear inclusions surrounded by a halo. Bronchopneumonia was evident, and neutrophils were present in the lumen of some bronchioles. The surrounding interalveolar septae were collapsed and thickened owing to infiltration with neutrophils and swelling of septal cells. There were no other lesions present.

In Lemur 5, the only microscopic lesion observed was hypertrophy and hyperplasia of bile duct epithelium. There were no abnormal findings in Lemur 8.

(C) *Histopathological findings in mice*

A second-passage mouse inoculated ic with isolate IbAn 24400 showed evidence of an acute necrotizing encephalitis and meningitis. The lesion, located in the parenchyma adjacent to the meninges, was one of malacia with extensive necrosis of cells. Intranuclear eosinophilic inclusion bodies were present in astrocytes. There was also evidence of a peritonitis and hepatitis.

A third-passage mouse inoculated ic with IbAn 24400 virus had lesions in the brain and skin. There was a generalized meningitis, encephalitis, and a necrotizing myelitis confined to the submeningeal white matter. Numerous extensive local areas of necrosis of the epidermis and dermis were present. Inclusions were evident in the nuclei of the squamous epithelial cells.

DISCUSSION

Herpesvirus hominis was isolated from the two adult lemur brains available for testing. The brain of the aborted foetus Lemur 4 did not yield virus.

The combination of the histopathologic findings, FA staining, CF test results, presence of CPE in HFDK cell cultures, and animal pathogenicity showed that the virus was a member of the herpesvirus group. Specific neutralization with *Herpesvirus hominis* MAF plus the virus's growth in human cells but not in monkey cells and its pathogenicity for adult as well as suckling mice, indicated that the virus was *Herpesvirus hominis* and not *H. simiae* or some other primate herpesvirus.

Since the other lemurs each showed some feature(s) of the clinical illness observed in Lemurs 1 and 2, it is assumed that all the lemurs were infected with herpesvirus.

Discovery of the actual source and time of introduction of the virus to these animals appears virtually impossible. Ever since the colony's establishment, the lemurs' diet had consisted of fruits and vegetables purchased on the open market, with occasional supplements of a boiled egg and bread scraps from the university kitchens. All primate diets were prepared in the same kitchen (see Fig. 1), and food handlers there gave no history of recent herpesvirus lesions, although they were not tested for antibody conversion. If one of the keepers had been excreting virus at the time of the move from cage F to cage J, Lemur 1 could have been exposed when handled during the move; dates of onset in the other animals suggest subsequent lemur-to-lemur transmission. Alternatively, after being relocated in cage J on the main thoroughfare through the Zoo, Lemur 1 may have contracted the virus from some member of the public. Since lemurs become quite excited when handled, it seems possible that the combination of the move to cage J and the continued proximity

there to crowds of people created a stressful condition in the animals that increased their susceptibility to infection.

ACKNOWLEDGMENTS

The Virus Research Laboratory, Ibadan receives support from the Rockefeller Foundation.

The authors acknowledge the technical service performed by Mr Dale Dondero of the Viral and Rickettsial Disease Laboratory, California State Department of Public Health.

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