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Detection of Rabies Virus Antibodies in Fruit Bats (*Eidolon helvum*) from Nigeria

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ABSTRACT: Fruit bats (*Eidolon helvum*) were collected from three different localities in Western Nigeria: Oti, Ibadan and Idanre. Fifty serum samples were analyzed using a modified rapid fluorescent focus inhibition technique against rabies, Mokola, Lagos bat and Duvenhage viruses. Twenty-five brain samples were screened for rabies and related lyssavirus antigens by direct fluorescent antibody microscopy and by an indirect fluorescent antibody technique with rabies anti-nucleocapsid monoclonal antibodies. All brain samples were negative. Two serum samples had relatively high anti-rabies activity; no neutralizing activity was detected against Mokola, Lagos bat or Duvenhage viruses.

Key words: Fruit bats, *Eidolon helvum*, rabies, lyssaviruses, monoclonal antibodies, serology, survey.

Rabies is of international public health significance. In Africa, much work remains to be done on its basic epidemiology (Boulger and Hardy, 1960). In Nigeria, as in most of the developing world, rabies is maintained primarily by dog to dog transmission. Much attention has been focused on rabies control for several decades but without major success (Boulger and Hardy 1960; Fagbami et al., 1981; Nawathe et al., 1981; Durojaiye, 1984). Rabies virus was recently isolated from clinically healthy but unvaccinated dogs in Nigeria (Aghomo et al., 1989), and a few cases were detected in wildlife during routine histopathological examination of carcasses (Okoh, 1976; Kasali, 1977; Oboegbulem et al., 1981). The ability of some wildlife to serve as a reservoir of rabies virus has been previously discussed (McMillan and Boulger, 1960; Tomori, 1980). However, firm evidence of potential factors responsible for the maintenance of rabies virus in Africa are poorly understood.

Boulger and Porterfield (1958) isolated Lagos bat virus from healthy fruit bats (*Eidolon helvum*) on Lagos Island, Nigeria. Although Lagos bat virus is morphologically and serologically related to rabies virus, it does not appear to be definitely associated with any natural pathological conditions in animals besides bats (Foggin and Swanepoel, 1985). Because the increasing frequency and widespread incidence of dog rabies is a cause for considerable concern in Nigeria, and because the epizootiology of Lagos bat and other lyssaviruses is poorly understood, we investigated the potential role of bats as reservoirs of rabies. This report details the initiation of a wildlife rabies survey in Nigeria in which the potential role of bats as reservoirs was examined.

Fruit bats (*E. helvum*) were randomly sampled from hunters, collected by shooting over a period of 2.5 yr at three different geographical locations, Oti, Ibadan and Idanre, in western Nigeria; the locations were within 6°3′ to 7°47′N and 2°30′ to 5°00′W. Thereafter, 2 ml of blood was collected from each bat by cardiac puncture. The blood was transferred into a vacutainer tube without anticoagulant. The blood samples were transported to the laboratory on ice packs. In the laboratory, samples were left at 26 C for 2 to 3 hr to clot. After clotting, the blood samples were centrifuged at 2,000 rpm for 10 min at 4 C. Decanted sera were stored at −85 C until analyzed. Fifty randomly chosen sera were analyzed for this particular study. The heads of the collected bats were also transported to the laboratory on ice. Brains were removed in the laboratory under aseptic conditions, placed in 5 ml polystyrene con-

tainers, and stored at −85 C prior to analysis.

From each of 25 original brain samples, three impressions were made on acetone-cleansed glass microscope slides. Smears were air-dried, fixed in acetone at 4 C for 30 min, and again air-dried. One set of impressions was stained with commercial fluorescein isothiocynate (FITC)-labeled anti-rabies ribonucleoprotein conjugate (Centocor Inc., Malvern, Pennsylvania 19355, USA), with a 1:2,000 dilution of Evan's blue (J. T. Baker Chemical Co., Phillipsburg, New Jersey 08865, USA), as counter stain (Wiktor et al., 1980). The other two impressions were each stained indirectly with rabies anti-nucleocapsid monoclonal antibodies 502-2 and 422-5 (Wiktor and Koprowski, 1978), and thereafter with FITC-labeled goat anti-mouse gamma globulin antibody (Cappel, Cooper Biomedical Inc., Malvern, Pennsylvania 19355, USA), as described (Wiktor et al., 1980). While still moist, the slides were viewed by fluorescent microscopy (Leitz Diaplan, Malvern, Pennsylvania 19355, USA) at 200× magnification.

The presence of rabies virus neutralizing antibodies (VNA) was determined by a modification (Reagan et al., 1983) of the rapid fluorescent focus inhibition test (Smith et al., 1973) using as in vitro challenge viruses rabies strain CVS-11, and African Lagos bat, Duvenhage, and Mokola viruses, adapted to cell culture as described (Rupprecht et al., 1987). The lower limit of VNA this particular assay could detect was 0.07 IU/ml. Standard human rabies immunoglobulin (HRIG) (Office of Biologics Research and Review, FDA, Bethesda, Maryland 20205, USA) was used as a positive serum control against rabies, and it contained 2.0 IU/ml of antibodies. Briefly, serial five-fold dilutions of sera were performed in Eagle's minimal essential medium (MEM) containing 10% fetal calf serum (MEM-10), 200 IU penicillin, 200 μg streptomycin and 29.23 mg of L-glutamine/ml (1:5 to 1:98,415) in a 96 well plate (Falcon, Oxnard, California 93030, USA). Thirty μl of a predetermined concentration of either CVS-11, Lagos bat, Duvenhage or Mokola viruses (approximately 100 $TCID_{50}$) were added to each dilution. The plate was incubated for 1 hr at 37 C in a 5.5% CO_2 atmosphere. After incubation 30 μl of indicator baby hamster kidney (BHK) cells (1.0×10^6/ml) were added to each well and thoroughly mixed. Ten μl from each well were put in duplicate wells in a Terasaki plate (Nunclon, Thomas Scientific, Swedesboro, New Jersey 08085, USA). The 96 well and the Terasaki plates were incubated as above for 24 hr. The Terasaki plate was washed 2× with PBS (pH 7.0) and drained before fixation in 80% cold acetone for 30 min. After fixation, the plate was dried at 37 C for 10 min, stained with 5 μl of commercial FITC-labeled anti-rabies ribonucleoprotein conjugate (Centocor, Inc.) and incubated as above for 30 min. The plate was rinsed 3× in distilled water and viewed while moist by fluorescent microscopy (Leitz Diaplan) at 200× magnification.

All 25 brain impressions were negative for rabies and related lyssaviruses. Two of the 50 serum samples had 2.0 IU/ml of rabies antibodies to CVS-11 virus, three samples had 0.7 IU/ml, four samples had 0.07 IU/ml and the remaining 41 samples had <0.07 IU/ml. All of the rabies VNA-positive sera came from the same area of collection (Ibadan). There was no detectable VNA (<0.07 IU/ml) to Lagos bat, Duvenhage or Mokola viruses compared to control wells receiving only virus.

The first documented cases of rabies in Nigeria were in 1912 and 1925, in human and dogs, respectively (Boulger and Hardy, 1960). Unlike most of Africa, wildlife play an important role in the epidemiology of rabies in Europe and the Americas. In Europe the principal vector is the red fox (*Vulpes vulpes*), while in the Americas, skunks (primarily *Mephitis mephitis*), raccoons (*Procyon lotor*), foxes (*Vulpes* sp.), wolves (*Canis lupus*), coyotes (*Canis latrans*) and bats (e.g., *Desmodus rotundus* and many insectivorous species) are the

main vectors (Bogel et al., 1976). In Nigeria, some historical rabies cases were diagnosed incidentally, during routine histopathological examinations of zoo animals such as caracal lynx (*Felix caracal*) (Okoh, 1976), civet (*Vivera civetta*) (Kasali, 1977) and chimpanzee (*Pan troglodytes*) (Oboegbulem et al., 1981), but these may have been exposed by contact with dogs. The experimental susceptibilty of other wildlife such as ground squirrels (*Xerus erythropus*) (McMillan and Boulger, 1960) and shrews (*Crocidura* sp.) (Tomori, 1980) has been documented, but poorly studied in the field. Some researchers contend that epidemiological studies may be greatly influenced by the attitudes of the local people (Nuru, 1973). However, the common belief among many Nigerian people that bites from some wildlife species such as bats, shrews and squirrels end in "madness" preceding death, presents an ancient recognition of sylvatic rabies among native people. The finding of rabies antibodies in the sera of some fruit bats in this study yields preliminary evidence for this ancient belief and suggests an active role by some wildlife in the maintenance of rabies in Nigeria. The three locations selected for this survey were established fruit bat colonies where the local people, primarily farmers and hunters, hunt them for food.

The relatively high levels of antibodies in the few bat sera analyzed suggest recent viral infection, especially since the levels are comparable with World Health Organization (WHO) recommendations for adequate antibody response levels in human serum after immunization. A finding of approximately 18% seroprevalence of rabies VNA warrants screening additional samples for analysis, provided they can be obtained from bat species in a manner that would not negatively impact their ecological status. It would be important to isolate and compare any bat virus strains with local canine rabies isolates to determine antigenic relationship versus relative independence, as suggested by other studies (Rupprecht et al., 1987). In addition, further studies are commencing on the role of other wildlife species, such as insectivores and rodents, in the epizootiology of rabies and related lyssaviruses in Nigeria.

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