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Antibodies to Viruses of Human Origin in Monkeys from Uganda (37955)

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Nonhuman primates are now commonly used as experimental animals. The selection of species for experimental studies is usually governed by a number of factors, including availability, cost, and problems of holding and handling. Expense and rarity of the larger animals, such as the gorilla (*Gorilla gorilla*), chimpanzee (*Pan* spp), and orangutan (*Pongo pygmaeus*), preclude their widespread use in biomedical experimentation. As a result of ease of procurement, Macaques are the animal of choice for many virological investigations. Of the cercopithecoids, the African green *Cercopithecus aethiops*) monkey is also widely used for biological studies and as a source of cells for tissue culture and vaccine production.

The expanded utilization of monkeys and apes in the experimental laboratory has not occurred without the recognition of the difficulties arising from extensive latent virus infections of all nonhuman primate species studied thus far. Recently, Kalter and Heberling (1) reviewed the evidence for infection of primates, mainly of captive apes and Macaques, with simian viruses and with other viruses primarily of human origin.

Except for the work of Kafuko *et al.* (2) and Munube *et al.* (3), few comparative virological data on the East African free-living (wild) and captive monkeys are available. This report presents data on the antibody patterns for human viruses other than arboviruses which can infect the respiratory and gastrointestinal tracts and for *Mycoplasma pneumoniae* in wild red-tail (*Cercopithecus ascanius*) monkeys commonly found in Uganda and in captive African green monkeys.

Materials and Methods. Monkey sera. Sera were kindly shipped frozen to us by Dr. G. M. R. Munube, East African Virus Research Institute, Entebbe, Uganda. They were collected from monkeys from three areas in Uganda: Kisubi and Bwamba (wild monkeys) and the Institute (captive monkeys originally captured from areas around Kisubi). Kisubi is only 5 miles away from the Institute. Bwamba is located in the Mountains of the Moon, 300 miles to the west of Kampala, the main city of Uganda. Wild monkeys were shot and bled in the field. All sera were separated and stored at $-20°$ until tested.

Antigens and immune sera. Prototype enteroviruses and homologous immune sera were provided by the Reference Reagents Branch, National Institute of Allergy and Infectious Diseases, Bethesda, MD. The myxovirus antigens, obtained from the Center for Disease Control, Atlanta, GA, included influenza A and B (soluble), parainfluenza 1 (strain C-39), parainfluenza 2 (strain Greer), parainfluenza 3 (strain C-243), mumps (strain Enders), and respiratory syncytial (RSV) (strain CCA-Long) viruses. Homologous immune sera were also provided by the Center for Disease Control. Other reagents were obtained from commercial sources or produced in our laboratory including adenovirus (group antigen), coronavirus (229E), *Herpesvirus*

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hominis (E38), Hepatitis B (Australian) antibody, and *Mycoplasma pneumoniae* (Lot i FH).

Hemagglutination-inhibition (HI) tests. Sera were kaolin treated and absorbed with human group O erythrocytes and then inactivated at 56° for 30 min. HI antibody was assayed employing microtiter techniques as described by Sever using 4 hemagglutination units of virus and 1 percent suspension of human group O erythrocytes (4). Each test included type-specific immune sera as a control. Monkey sera were tested at 1/10 and 1/20 dilutions. Serum–virus mixtures were incubated overnight at 4°, 0.05 ml of red blood cells (RBC) added, and then incubated at either 4 or 37° for 1 hr. Sera with titers (reciprocal of dilution) of 10 or greater were considered positive.

Serum neutralization tests. The micromethod described by Kyriazopoulou and Bell (5) was employed. Briefly, 0.05 ml of virus suspension, containing 100 TCD_{50} per 0.025 ml, was mixed with an equal volume of serum diluted 1/5 and previously inactivated at 56° for 30 min. The serum–virus mixtures were incubated at 37° for 1 hr, mixed with 0.15 ml of HEp-2 cell suspension, and dispensed in microplate wells. The plates were sealed with transparent cellophane tape and incubated at 37° for 4 days. Included in each test were serum controls, cell suspension, simultaneous virus titration, and type-specific immune sera.

Complement-fixation (CF) tests. Microtiter procedures employing 4 units of antigen, 1.8–2.0 units of complement, and overnight fixation at 4° were employed. Sera were inactivated at 56° for 30 min. Anticomplementary activity present in a few sera were removed by absorption with mouse liver powder as described by Rapp *et al.* (6).

Results. Sera from 52 red-tail and 31 African green monkeys were examined for the prevalence of viral antibodies (Table I). Sera positive for antibodies to mumps, RSV, coronavirus and *M. pneumoniae,* and Hepa-

TABLE I. Survey of Antibodies to Viruses and *M. pneumoniae* and of Hepatitis B Antigen in Wild Red-Tail and Captive African Green Monkeys.

Agent[a]	Serologic test[b]	Species of monkeys and place of origin: Red-tail Bwamba		Red-tail Kisubi		African green Captive		All	
M. pneumoniae	CF	2/30[c]	(6.6)	0/13	—	6/27	(22.2)	8/70	(11.4)
Influenza A	CF	2/30	(6.6)	2/13	(15.3)	1/27	(3.7)	5/70	(7.1)
Influenza B	CF	1/30	(3.3)	0/13	—	1/21	(3.7)	2/70	(2.8)
Parainfluenza 1	CF	3/30	(9.9)	3/13	(23.0)	2/27	(7.4)	8/70	(11.4)
Parainfluenza 2	CF	7/30	(23.3)	3/13	(23.0)	8/27	(29.6)	18/70	(25.7)
Parainfluenza 3	CF	4/30	(13.3)	3/13	(23.0)	9/27	(33.3)	16/70	(22.8)
Mumps	CF	2/30	(6.6)	0/13	—	7/27	(25.9)	9/70	(12.8)
Adenovirus	CF	1/30	(3.3)	1/13	(7.6)	2/27	(7.4)	4/70	(5.7)
Respiratory syncytial	CF	6/30	(19.9)	1/13	(7.6)	3/27	(11.1)	10/70	(14.3)
Coronavirus (229E)	CF	3/30	(9.9)	1/13	(7.6)	2/27	(7.4)	6/70	(8.6)
Herpesvirus hominis	CF	6/30	(19.9)	0/13	—	17/27	(62.9)	23/70	(32.8)
Hepatitis B	CF	6/30	(19.9)	0/13	—	1/27	(3.7)	7/70	(8.4)
ECHOvirus 7	HI	1/37	(2.7)	0/15	—	17/31	(54.8)	18/83	(21.6)
ECHOvirus 11	HI	1/37	(2.7)	1/15	(6.6)	3/31	(9.6)	5/83	(6.0)
ECHOvirus 12	HI	0/37	—	0/15	—	4/31	(12.9)	4/83	(4.8)
ECHOvirus 19	HI	0/37	—	0/15	—	2/31	(6.4)	2/83	(2.4)
Coxsackie B-5	HI	0/37	—	1/15	(6.6)	2/31	(6.4)	3/83	(3.6)

[a] All sera were negative for neutralizing antibodies to poliovirus types 1, 2, and 3, Coxsackie B-3 virus, and ECHOvirus 6.

[b] CF, complement fixation; HI, hemagglutination-inhibition.

[c] Number of animals seropositive/number of animals tested. ( ) Percentage.

titis B antigen all had titers between 8 and 128 (Table II).

Influenza. Overall, 7.1 and 2.8% of the 70 monkeys tested had antibodies to influenza A and B viruses, respectively. The occurrence of the antibody to these viruses seemed to be independent of place of origin of the animals. Two Kisubi monkeys were found to have antibodies to influenza type A and none to type B.

Parainfluenza. The prevalence of antibody to parainfluenza 1, 2, and 3 viruses in wild and captive monkeys varied from 7.4 to 33.3%. In red-tail monkeys from Kisubi, antibody to each of these viruses was present in approximately 1 of 4 animals. A similar proportion of monkeys from Bwamba (wild) and the Institute (captive) had antibodies to two parainfluenza viruses, types 2 and 3; parainfluenza 1 virus antibody was detected in less than 1 in 10 animals.

Mumps. Monkeys from Kisubi lacked CF antibodies to mumps virus. Only 2 animals (6.6%) from Bwamba had mumps antibodies. By comparison, the highest percentage of antibodies to mumps virus was found in the Institute captive monkeys (25.9%).

RSV. The occurrence of antibodies to RSV was highest in Bwamba monkeys; 6 of 30 animals (19.9%) were positive. Only 1 of 13 Kisubi and 3 of 27 Institute monkeys had RSV antibodies.

Adenovirus. Only 4 (5.7%) of 70 monkeys tested were positive for adenovirus CF antibodies. Infection was independent of the area of origin of the monkeys.

Coronavirus. Six (8.6%) of 70 monkeys were positive for coronavirus. As for adenovirus, the distribution of positive animals was independent of the locality of monkeys.

Herpesvirus hominis. The prevalence of antibodies to *H. hominis* was markedly higher in captive than in wild monkeys, 62.9% compared to 19.9%. Antibodies to this agent were not detected in any Kisubi monkeys tested.

Hepatitis B Antigen. Six (19.9%) Bwamba and 1 (3.7%) Institute monkeys were positive for Hepatitis B antigen. Sera obtained from Kisubi monkeys were negative.

Enteroviruses. HI antibodies to enteroviruses tested were found almost exclusively in captive monkeys. Interestingly, the number of sera positive for ECHOvirus type 7 antibodies (54.8%) was especially high in Institute monkeys. None of 83 monkeys in the test group possessed neutralizing antibodies to poliovirus types 1, 2, and 3, Coxsackie B-3, and ECHOvirus type 6.

Mycoplasma pneumoniae. Antibodies to *Mycoplasma pneumoniae* were found in 2 (6.6%) Bwamba and in 6 (22.2%) Institute monkeys. Monkeys from Kisubi were seronegative.

Discussion. The more frequent occurrence of antibodies to *Herpesvirus hominis,* mumps virus, and several human enteroviruses in captive Institute African green monkeys compared to wild red-tail monkeys from Kisubi and Bwamba, Uganda, suggests that monkeys held in laboratory animal colonies can readily acquire infections with viruses of human origin. The similar proportion of captive and wild monkeys with antibodies to RSV, influenza, parainfluenza, adenovirus, and coronavirus leaves open the question whether these antibodies reflect prior naturally occurring infection with simian viruses which share antigens with

TABLE II. Levels of Antibodies for Selected Agents and of Hepatitis B Antigen in Monkeys.

Agent	Number positive	No. of sera positive at dilution			
		1/8	1/16	1/32	1/128
Mumps	9	3	4	2	0
Respiratory syncytial	10	5	1	3	1
Coronavirus (229E)	6	3	3	0	0
M. pneumoniae	8	0	3	5	0
Hepatitis B	7	4	3	0	0

human viruses or whether monkeys contract human viruses upon contact with man.

The infection of captive monkeys through human contacts has important consequences for investigators employing primates, especially in experiments with viruses. Antibody present as a result of infection acquired in captivity precludes use of the animal for production of specific immune antiserum or vaccine development trials. Such acquired virus infections could interfere with employing the monkeys as models in experimentally induced infections. Interferon formed in response to an acquired infection might also influence the course of infections of monkeys in experimental situations.

Of the virus infections apparently transmitted to captive monkeys, the high proportion of animals with antibody to *Herpesvirus hominis* reflects the ubiquitous occurrence of this group of viruses. Most monkeys in close contact with man and other simians possess *Herpesvirus hominis* antibody (7). Recent serologic studies by previous workers (8, 9) documented the widespread distribution in primates of complement fixing and immunofluorescent antibody to herpesvirus. Natural *Herpesvirus hominis* infection of a gibbon (*Hylobates lar*) has been reported by Emmons and Lennette (10). Ohwada and coworkers (11) found that 65.1% of *C. aethiops* and 69.9% *M. irus* possessed antibody to *Herpesvirus hominis.*

Of particular interest was the finding that captive African green monkeys had a high rate of ECHOvirus type 7 infection, whereas only one wild monkey possessed antibody to this virus. These findings in captive monkeys are consistent with the epidemic character of ECHOvirus type 7 infection in Africa (1). The prevalence of several other enterovirus infections was also more common in captive monkeys. Antibody to poliovirus types 1, 2, and 3, however, was not detected in monkeys despite the fact that there was a recent outbreak of type 1 paralytic poliomyelitis among the residents of Uganda (12).

Antibody to mumps virus in nonhuman primates has been previously demonstrated (13, 14). Our finding of more seropositive animals among captive than wild monkeys supports the concept that mumps virus infections can be readily acquired through human contacts.

The antibody patterns of influenza and parainfluenza viruses exhibited by captive monkeys did not differ from those of wild animals, except that more wild Bwamba monkeys were seropositive for parainfluenza type 1 virus than Institute monkeys. A predominance of parainfluenza type 3 seropositive animals were reported by Shah and Southwick (15) and Hsiung *et al.* (13). Our findings suggest that the occurrence of parainfluenza 2 and 3 virus infections in monkeys varies with the geographic origin of the animals. The prevalence of influenza A and B virus infections in Ugandan monkeys was low and independent of local origin of the animals.

Adenovirus and coronavirus antibodies were detected in a few monkeys. The rate of adenovirus antibody-positive monkeys was similar to that reported by Bhatt and coworkers (16). Rapoza and Atchinson (17) examined sera derived from a variety of primates for antibody to the adenovirus group antigen. All the sera tested except those obtained from Patas monkeys were positive for such antibody. The occurrence of coronavirus infections in monkeys has not been previously reported. Further investigation is required to answer the question whether monkeys acquire this viral infection through human contact or whether the presence of antibody represents a cross-reaction with a simian coronavirus.

Neutralizing antibody to RSV was detected in only 1 of the 46 monkey sera tested by Shah and Southwick (15). Kalter and coworkers reported similar results (18). By contrast, 1 in 4 wild monkeys from the Bwamba region possessed complement-fixing antibodies to RSV, suggesting that natural infection with this virus or a related agent occurred in Ugandan monkeys from specific geographic areas.

In their systematic investigation of the mycoplasmal flora of monkeys, Del Giudice and coworkers isolated 70 strains of oral and genital origin (19). Interestingly, no strain of *M. pneumoniae* was isolated. The nonhuman primates who harbored the same

mycoplasmas as man included *C. aethiops, M. mulatta,* the chimpanzee, the gorilla, and the orangutan. Kalter found antibody to *M. pneumoniae* in all species tested (20). *M. pneumoniae* antibody was detected among 2 of our 3 groups of monkeys.

That hepatitis B antigen was detected in sera of some Ugandan monkeys indicates its widespread distribution and raises the question of whether or not these infections occurred in the wild or after association with man. More importantly, such positive monkeys can serve as a source of infection for laboratory workers. Consequently, all primates should be tested for hepatitis B antigen before release from quarantine and use in the laboratory.

Summary. Sera from 52 red-tail (wild) and 31 African green (captive) monkeys were examined for the prevalence of viral antibodies. Antibodies to *Herpesvirus hominis,* mumps virus, certain human enteroviruses, and *M. pneumoniae* occurred at a greater frequency in captive monkeys, suggesting that nonhuman primates acquire these infections through contact with humans. Antibodies to parainfluenza viruses, adenoviruses, coronaviruses, influenza A and B viruses, and respiratory syncytial virus occurred as often in captive as in wild monkeys. All sera were negative for neutralizing antibodies to poliovirus types 1, 2, and 3, Coxsackie B-3 virus, and ECHO-virus type 6. Wild monkeys from the Bwamba region were found to have a high rate of hepatitis B infections; few captive monkeys were positive for this infection.

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