ESTABLISHMENT OF A COLONY OF NONHUMAN PRIMATES (AOTUS LEMURINUS GRISEIMEMBRA) IN COLOMBIA

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The owl monkey Aotus lemurinus, valued as a model for research on human malaria, is found in much of South America, including forested areas of northern Colombia. This article describes establishment of an Aotus colony by Colombia’s National Institute of Health, the animal health problems encountered, and the steps taken to achieve a reasonable measure of success.

Introduction

Primates were first used as experimental research models in yellow fever studies initiated at the laboratories of Brazil’s National Yellow Fever Service and at the Gorgas Memorial Laboratory in Panama during the 1920s (1). Since that time, many primate species have been used to study infectious and parasitic diseases, including viral hepatitis, herpesvirus infections, leishmaniasis, and malaria (2-6).

In 1966 Porter and Young (4) showed that the owl monkey species Aotus lemurinus was susceptible to experimental malaria infections, developing the disease with pathologic characteristics similar to those observed in humans. Since then, this primate has become the subject of choice for research on malaria immunology and chemotherapy (7-8).

Colombia’s National Institute of Health (Instituto Nacional de Salud—INS) began malaria immunology studies using Aotus specimens in 1979 as a means of evaluating different immunogens obtained from Plasmodium falciparum that could be used in possible antimalarial vaccines. The purpose of this article is to describe the adaptation and maintenance of an INS colony of

Aotus lemurinus griseimembra in closed environments.

Materials and Methods

Aotus is the only nocturnal primate genus whose members are native to the Americas. It is found in almost all forested areas of South America and Panama, in widely varied habitats ranging from the Amazon jungles to the temperate forests of the Andean highlands. Its diet consists mostly of plant material such as fruits, seeds, tender leaves, and (to a lesser degree) foods of animal origin such as arthropods, bird eggs, and young birds. Members of the genus typically live in family groups consisting of the parents and one or two young offspring.

The INS Aotus colony was started in Bogotá in July 1979 with an initial complement of 57 specimens brought in from the San Marcos township near Sucre in the department of Bolivar. This area, in northern Colombia, is situated between the San Jorge and Cauca rivers. Two additional lots of monkeys were brought in later from this same area. One, consisting of 108 monkeys, was obtained in August 1979; and the other, consisting of 51, was obtained in April 1981. The first of these three lots was purchased from a local hunter who delivered them to INS headquarters in Bogotá. The second and third lots were captured by teams of residents from the San Marcos area who were hired and supervised by INS researchers.
A family group of *Aotus lemurinus griseimembra* monkeys at the National Institute of Health colony in Bogotá.

**Circumstances of Capture**

All the monkeys captured were found in the area of the San Marcos township between the San Jorge and Cauca rivers—a low-lying region 100 to 300 meters above sea level that is often flooded in the June-December rainy season. Forests in this area are gradually being reduced to small isolated stands. Most of the open land is currently used for cattle-raising and, to a lesser degree, for rice and corn cultivation. The average annual temperature is 26°C, and the average relative humidity is 80%.

Traditional local methods were used to catch the monkeys. These methods are as follows: A team of hunters, usually consisting of seven to 10 individuals familiar with the area, fans out through the woods searching for specimens in the hollow trees and vines that are the most likely hiding places. When a group of *Aotus* (usually consisting of three or four individuals) is sighted, trees and bushes within a radius of 10 meters around the site are cut down to eliminate possible escape routes. The monkeys are then forced to jump to the ground (or in the rainy season into the water), where they are caught by the hunters.

Regarding the monkeys in the second and third lots, those captured by the various teams of hunters were brought together and held in one place until the required number was assembled. The long and exhausting trip to the laboratory then proceeded in stages by canoe, mule-back, motor vehicle, and airplane. Since this took some 30 to 36 hours, the monkeys were sedated with levomepromazine (5 mg/kg) com-
bined with oxytetracycline (10 mg/kg) and multivitamins and minerals (1 gr/kg) in 5 cc of distilled water. From the time they were captured, and throughout the journey to the laboratory, the monkeys were closely monitored by a veterinarian.

**Handling and Maintenance**

An individual clinical history was established for each *Aotus* entering the colony. This history included the individual’s place of origin, described its phenotype, and recorded the results of a detailed clinical examination that included a general physical examination, determination of vital signs, and evaluation of blood and stool samples for pathogenic microorganisms. These procedures marked the start of a period of careful observation lasting about eight weeks.

During the first week, the monkeys were immunized with intramuscular 0.5 cc injections of *Herpes simplex* and *H. tamarinus* vaccines administered to the outside of the left or right thigh. These vaccines were obtained through the Pan American Health Organization from the New England Primate Center in Boston, Massachusetts, U.S.A.

After receiving identifying tattoos, the animals were paired by phenotype, compatibility, and general condition. Each pair was housed in a square metal cage 70 cm on a side that was constructed with angle irons and galvanized 2.5 cm wire mesh; each cage contained a central perch. The monkeys’ behavior, together with their food and water intake, was monitored daily.

Each monkey’s weight and temperature were recorded at weekly intervals. (Air conditioning and humidifiers kept the temperature in the cages at about 25°C and the relative humidity at 70-80%.)

To adapt the monkeys to normal laboratory working hours, the day-night cycle was reversed by means of artificial lighting. That is, the cage area was lit with red light for 11 hours daily (from 6 a.m. to 5 p.m) and was lit with white light for the remaining 13 hours. This caused the monkeys to be active in the red-light period and to rest during the white-light period. The white light was provided by fluorescent tubes and the red by similar ones covered with red cellophane.

All cleaning and maintenance work for the colony is performed in the morning hours. This includes daily hosing and weekly disinfection of the monkeys’ cages, trays, food containers, and water vessels. Laboratory procedures involving handling are also performed in the morning. All parts of the animal facilities are routinely disinfected twice a week with antiseptic solutions based on phenol, iodine, and other ingredients. The bacterial content of all sections of the vivarium is checked every three months.

After the cleaning chores are finished, the monkeys are fed a serving of whatever appropriate fruits happen to be in season—such as bananas, guavas, papayas, pineapples, or mangos—and five or six biscuits of a commercial product (Purina Monkey Chow® containing 15-25%
protein, 5% fat, vitamins, and minerals). This latter food is provided twice every 24 hours; water is provided ad libitum.

Laboratory Tests and Determination of Biological Constants

The proper taxonomic classification of the monkeys was determined in accordance with their place of origin (9, 10) and by phenotype and karyotype studies (11, 12). Karyotyping was performed by the INS genetics laboratory using the white cell culture technique of Moorhead and colleagues (13). Hematology values were determined by means of an automatic Coulter Counter (Model S) at the Bogotá Central Military Hospital’s hematology laboratory. Parasitologic and microbiologic studies were performed by the appropriate INS laboratories through direct examination and culture of stools, blood samples, and pharyngeal and conjunctival secretions (14, 15).

Yellow fever antibodies were detected by hemagglutination-inhibition testing (16) at the INS virology laboratory. Tuberculosis tests were accomplished by intradermal injection of 0.1 ml of RT-23 tuberculin (2 IU) in the abdomen of each animal, with the transverse diameter of the resulting reaction at the injection site being read at 72 hours.

Detailed autopsies were performed on any animals that died during the quarantine or maintenance periods. Tissues fixed in buffered formalin were sent to the INS pathology laboratory for histopathologic studies and, in some instances, examination by electronic microscope.

Results

Phenotype and Karyotype

The dorsal fur of the colony's Aotus monkeys is an agouti-patterned (grizzled) greyish-brown. Pale yellow hair covers the abdomen and chest; this color extends out as far as the clavicle (sometimes as far as the chin) and also extends partly over the inner surfaces of the arms and legs (sometimes going as far as the elbows and knees). When the pale yellow fur does not continue up to the chin, the fur at the front of the neck is grey of a somewhat lighter shade than the fur of the back. The color of the rest of the arms and legs, and also the hands and feet, is a variable grizzled brown—in some cases so light as to appear almost yellow and in others so dark as to seem almost black. The tail fur is greyish at the base and starts to darken at the midpoint; the last third of the tail is black. This phenotype, with certain individual variations, is similar to that of the “B” phenotype described by Ma and colleagues (11).

The karyotype of the colony animals is polymorphous, with diploid numbers of 54, 53, and 52 chromosomes corresponding to karyotypes II, III, and IV described by Ma et al. (11, 12).

The phenotype and karyotypes of these specimens (see Table 1) are similar to those of Aotus lemurinus griseimembra (a synonym for Aotus trivirgatus griseimembra) (17) that is native to this part of northern Colombia (9, 10).

<table>
<thead>
<tr>
<th>No of monkeys</th>
<th>Phenotype</th>
<th>No of chromosomes</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>58</td>
<td>B</td>
<td>54</td>
<td>II</td>
</tr>
<tr>
<td>60</td>
<td>B</td>
<td>53</td>
<td>III</td>
</tr>
<tr>
<td>10</td>
<td>B</td>
<td>52</td>
<td>IV</td>
</tr>
</tbody>
</table>

Laboratory Tests

Hematologic values were obtained for 57 specimens six to eight months after they were brought in from the field to the laboratory (Table 2). Testing of 90% of the colony for yellow fever antibodies produced negative results. The results of tuberculosis tests performed on 70% of the population also proved negative.
Table 2. Results obtained by examining blood specimens from 57 *Aotus lemurinus griseimembra* monkeys in the second lot captured. The blood specimens were taken six to eight months after their capture. The specific counts indicated were obtained with a Coulter Counter at the Central Military Hospital in Bogotá.

<table>
<thead>
<tr>
<th>Item evaluated</th>
<th>Average (X) ± 1 standard deviation</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red cells (10⁶/mm³)</td>
<td>6.55 ± 0.60</td>
<td>5.22–7.93</td>
</tr>
<tr>
<td>Mean corpuscle volume (fl)</td>
<td>80.20 ± 5.06</td>
<td>70–95</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>52.80 ± 5.61</td>
<td>36.8–63.1</td>
</tr>
<tr>
<td>Hemoglobin (g/100 ml)</td>
<td>18.73 ± 1.53</td>
<td>14.4–24.1</td>
</tr>
<tr>
<td>Platelets (10⁹/mm³)</td>
<td>224.36 ± 77.20</td>
<td>102.4–460.0</td>
</tr>
<tr>
<td>Leukocytes (10³/mm³)</td>
<td>14.08 ± 3.82</td>
<td>7.7–24.8</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>38.38 ± 12.25</td>
<td>20–76</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>51.07 ± 13.04</td>
<td>26–74</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>3.68 ± 2.33</td>
<td>1–11</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>7.04 ± 4.79</td>
<td>1–19</td>
</tr>
<tr>
<td>Basophils (%)</td>
<td>0.01 ± 0.13</td>
<td>0–1</td>
</tr>
</tbody>
</table>

**Morbidity and Mortality**

All of the animals in the first group, which had been caught and delivered to the INS by local hunters, died because of an epizootic caused by type 1 *herpesvirus hominis* within two weeks of their arrival in Bogotá. Infected in the field by their captors, at the time of their arrival in the laboratory they showed signs of unilateral or bilateral keratoconjunctivitis, at times of a purulent nature, with extensive rhinorrhea, anorexia, hypothermia, loss of equilibrium, and ataxia. The virus was identified by means of histopathologic, electron microscopy, and direct immunofluorescence procedures (5).³

The results of parasitologic tests performed on 100 stool specimens appear in Table 3. The parasites most frequently found were *Giardia sp.*, *Enterobius sp.*, and *Trichomonas sp.* Examination of peripheral blood for parasites (including *Plasmodium*, *Trypanosoma*, and *Filaria*) yielded negative results. However, *Trypanosoma sp.* was found in peripheral blood specimens from three *Aotus* that had undergone experimental splenectomies. The trypanosomes, isolated by xenodiagnosis and growth in vitro, are now in the process of being identified.

Also, a histopathologic study performed on one animal yielded a finding of systemic toxoplasmosis, and this finding was confirmed by the indirect immunofluorescence test.

A microbiologic study of the 100 stool specimens mentioned above revealed the presence of various enterobacteria—including *Yersinia enterocolitica* in five specimens.

Medical problems afflicting the monkeys of lots two and three after their arrival at the laboratory included the following:

Most likely as a result of malfunctioning air conditioning equipment, 80 animals developed a picture of keratoconjunctivitis with epizootic characteristics. No deaths occurred, and the crisis was surmounted in about 10 days with topical administration of a 5% boric acid solution to each monkey. Some of the possible causative agents isolated included *Streptococcus faecalis*, *Proteus sp.*, *Bacillus subtilis*, and *E. coli*.

In addition, a respiratory disorder involving dyspnea, rhinorrhea, cough, and anorexia was found in a group of 30 specimens sharing the same room. A sampling of pharyngeal smears from 15 of them revealed the presence of α-hemolytic *Streptococcus*, *Staphylococcus albus*, *S. aureus*, and *E. coli*. Following treatment with erythromycin (10 mg/kg), bronchodilators, orciprenaline (0.1 mg/kg), and vitamin C (20 mg/kg), all of the monkeys recovered.

Laceration, necrosis, and sepsis—all probably of a traumatic origin—were observed at the

³Liver tissue samples fixed in neutral formaldehyde and prepared for light microscopy were found to contain intranuclear Herpes-type inclusions. The tissues involved were washed with distilled water and phosphate buffer (pH 7.2) and were later prepared for electron microscopy by standard methods.

Table 3. Intestinal parasites detected in 100 stool specimens obtained from captured *Aotus lemurinus griseimembra*.

<table>
<thead>
<tr>
<th>Parasite</th>
<th>% of monkeys positive for indicated parasite</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Giardia sp.</em></td>
<td>18</td>
</tr>
<tr>
<td><em>Enterobius sp.</em></td>
<td>4</td>
</tr>
<tr>
<td><em>Trichomonas sp.</em></td>
<td>3</td>
</tr>
<tr>
<td><em>Enterobius vermicularis</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Strongyloides stercoralis</em></td>
<td>2</td>
</tr>
<tr>
<td><em>Trichuris sp.</em></td>
<td>2</td>
</tr>
</tbody>
</table>

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Table 4. Histopathologic findings obtained from examination of 26 *Aotus lemurinus griseimembra* monkeys that died in the INS colony.

<table>
<thead>
<tr>
<th>Cause of death reported</th>
<th>No of monkeys dying</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
</tr>
<tr>
<td>Respiratory tract infections.</td>
<td></td>
</tr>
<tr>
<td>Bronchopneumonia:</td>
<td></td>
</tr>
<tr>
<td>Bacterial pneumonia</td>
<td>4</td>
</tr>
<tr>
<td>Aspiration pneumonia</td>
<td>2</td>
</tr>
<tr>
<td>Cardiac insufficiency, pulmonary edema</td>
<td>3</td>
</tr>
<tr>
<td>Chronic interstitial nephritis</td>
<td>2</td>
</tr>
<tr>
<td>Hepatitis:</td>
<td></td>
</tr>
<tr>
<td>Fat metamorphosis</td>
<td>1</td>
</tr>
<tr>
<td>Type undetermined</td>
<td>1</td>
</tr>
<tr>
<td>Septicemia</td>
<td>2</td>
</tr>
<tr>
<td>Other causes:</td>
<td></td>
</tr>
<tr>
<td>Electrocuton</td>
<td>1</td>
</tr>
</tbody>
</table>

tips of the tails of 70% of the population. Treatment consisted of surgery and antibiotics (oxytetracycline at 10 mg/kg).

Of the 159 monkeys in lots two and three that were captured by a team of local residents supervised by a veterinarian and a primatologist, 26 (16%) died within a period of two and a half years. The histopathologic findings following these deaths are shown in Table 4. As may be seen, the causes of death found most frequently were bronchopneumonia, cardiopulmonary disorders, and septicemia.

**Discussion and Conclusions**

Obtaining primates in top condition for biomedical research requires an appropriate organizational and technical structure. More specifically, it requires selection of appropriate capture areas, development of adequate supply facilities, participation by suitable qualified personnel, and application of sound methods for capturing the animals, maintaining them in the field, transporting them to the laboratory, and sustaining them effectively after they arrive.

Despite its primitive nature, the traditional method of catching *Aotus* is effective. The merits of this brush-clearing method are debatable, however, since on the one hand it damages the setting, while on the other it provides a secondary environment favoring small primates—so long as it is applied in a limited way to a small area (18). Regardless of these points, however, it clearly seems advisable to standardize capture methods that reduce both changes in the natural environment and possible damage of the captured primates to a minimum.

We must also emphasize the importance of providing professional veterinary care for the captured animals as soon as they are caught, during their maintenance in the field, and throughout their trip to the laboratory. In our own case, this practice appears to have been largely responsible for lowering both morbidity and mortality—particularly the latter, which fell from 100% of the animals in the first group (captured and delivered by a local hunter) to 5% in the last two groups (captured by the Institute’s work team).

The initial observation period in the laboratory is also crucial, for it is then that the animals begin their adaptation to a closed environment. In particular, provision of gentle, considerate treatment and a proper diet is essential. Moreover, during this period the monkeys must be carefully evaluated with regard to major biological parameters, so as to provide a point of reference making it possible to assess their subsequent development in captivity.

Regarding diet, it has been observed that a technically correct diet has a direct and positive impact on physical health, reproductive ability, and adaptation (19). In our case, when food prepared at the Institute was used, we noted that one group of specimens suffered alteration of the 2:1 ratio of calcium to phosphorus and experienced an exaggerated increase in alkaline phosphatase values. These findings—which appear similar to ones associated with bone malformation, spontaneous fractures, and dentition problems—pointed to an obvious nutritional imbalance. After the commercially prepared food now being used (Monkey Chow®, made by Ralston Purina of St. Louis, Missouri) was substituted, these values returned to normal levels within eight months.
The karyotypes and general phenotype of the northern Colombian animals collected are similar to those reported by Ma et al. in 1976 (11, 17), which has led us to conclude that they are members of the subspecies *Aotus lemurinus griseimembra* (17).

The hematologic values found in 57 animals six to eight months after capture indicated erythrocyte, hematocrit, and hemoglobin levels exceeding those reported by other authors (20-22). This was probably due to the altitude, since the colony is located at 2,650 meters above sea level. Also, the white cell counts tended to resemble those reported by Porter (20), who was examining animals that had spent one to two years in captivity. This was probably caused by the same factors noted by Porter, whose findings reflected exposure in the laboratory to higher bacterial loads than are commonly found in these monkeys' natural habitats.

The colony's temporary keratoconjunctivitis infection may have been prompted by imperfections in the air conditioning system—with a consequent rise in temperature, lack of fresh air, and low humidity.

The source of the *Yersinia enterocolitica* infection found in stool specimens has not yet been determined, but the animals may have acquired it in their natural habitat. None of the infected primates showed any of the symptoms associated with this enterobacteria, and this is the first time its presence has been reported in Colombia in an *Aotus* colony.

The detection of trypanosomes in several of the monkeys following splenectomies showed the importance of using serologic techniques to find blood parasite (*Trypanosoma, Filaria, Plasmodium*) infections during the quarantine period and before any experimental procedures are begun.

The high percentage of caudal necrosis cases may have been caused by the monkeys' tails rubbing against abrasive material on the floors and coming into contact with contaminants deposited there. This finding suggests that the design and materials of the cages should be adjusted to the maintenance requirements for this species.

Despite all the aforementioned problems, however, it seems evident that our experience with the colony to date has been successful. Overall, that experience has served as a working demonstration of the fact that constant monitoring of the animals' health and general condition, provision of a proper diet, and reduction of stress through careful and gentle handling permits these primates to be maintained in optimal condition for various types of malaria research.

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Special thanks go to the Pan American Health Organization and to Dr. Manuel Moro for helping to obtain the *Herpes simplex* and *H. tamarinus* vaccines and the Purina Monkey Chow®, as well as for their invaluable technical support.

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SUMMARY

The owl monkey Aotus lemurinus, a preferred experimental animal for various kinds of malaria research, is found in much of South America, including forested areas of Colombia.

In 1979 Colombia's National Institute of Health (INS) established an Aotus colony with 57 monkeys captured and delivered by local hunters. These all died of a Herpesvirus hominis epizootic within two weeks of their arrival, the causative agent presumably having been passed to them by their captors.

Subsequently, the INS hired teams of hunters and provided professional supervision by a veterinarian and primatologist from the time of capture. Lots of 108 and 51 Aotus lemurinus griseimembra were obtained by this approach in August 1979 and April 1981, respectively. Overall, experience to date with these latter lots of monkeys has been successful.

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SEXUALLY TRANSMITTED DISEASES IN CANADA

According to Canada's 1982 National Report on Sexually Transmitted Diseases (STDs), reportable STDs accounted for 69% of all notifiable diseases in Canada that year. Gonorrhea ranked first with 53,076 cases or 96% of the total reported STDs. The overall reported incidence rate for gonorrhea was 216 cases per 100,000 population, which represented a 7% decrease from 1981. Rates dropped in all provinces and territories in 1982. Persons 15-29 years of age accounted for 76% of the total number of cases. The male-to-female ratio for those over 20 years of age was 1.6:1, while the ratio for those under 20 was 1:1.7. Fifty-eight cases of gonorrhea were reported in children one to nine years old.

The first Canadian isolation of penicillinase-producing *N. gonorrhoeae* was reported in 1976. Since that time, 175 isolates have been submitted to the Canadian Bureau of Microbiology, Laboratory Center for Disease Control. The 73 strains reported in 1982 accounted for 0.14% of all reported gonococcal infections. This represents an increase of 22% over 1981. The majority of these infections were contracted in Asia.

There were 2,288 cases of syphilis reported in Canada during 1982, of which 966 (42%) were infectious. Rates of infectious syphilis declined or remained static in all age groups except females 15-19 years old. There were also 1,222 cases of latent syphilis reported in 1982. The overall incidence was 9.3 cases per 100,000, a decline of 21% from 1981.

There were 6,224 laboratory reports of herpesvirus infections and 1,735 of chlamydial infections in Canada in 1982.