RESEARCH REPORT

Clinical Examinations on Crab-eating Macaques in Mauritius*

KIYOAKI MATSUBAYASHI, SHUNJI GOTOH, YOSHI KAWAMOTO, *Kyoto University*
TSUYOSHI WATANABE, *Sugiyama Jogakuen University*
KEN NOZAWA, *Chukyo University*
MASAO TAKASAKA, TOYOKO NARITA, *Tsukuba Primate Center for Medical Science*
OWEN GRIFFITHS, and MARY-ANN STANLEY, *Bioculture (Mtius) Ltd.*

ABSTRACT. Clinical inspections were carried out in order to obtain sufficient information concerning the physiological characteristics and states of natural infection by various pathogenic agents in the population of crab-eating macaques (*Macaca fascicularis*) on Mauritius. The hematological and plasma biochemical values were within the normal ranges, showing no morbid signs. The intestinal parasitic appearance was so simplified that only *Strongyloides* and *Trichuris* were noted as helminthic findings. All of the monkeys examined were free from enteropathogenic organisms such as *Shigella* and *Salmonella*, and were negative for measles, herpes simplex type 1, simian immunodeficiency virus/MAC, and SV5 antibodies. These data suggest that the macaques in Mauritius are considerably spared from natural infections by various pathogenic agents.

Key Words: Crab-eating macaque; Mauritius; Isolation effect; Parasitic fauna; Viral antibody.

INTRODUCTION

Crab-eating macaques (*Macaca fascicularis*), which widely inhabit the continental and island regions of Southeast Asia from Myanmar to Timor, sometimes live in remote habitats to which they were previously transplanted by human beings. One such case is the population introduced in the early 1900's to Angaur Island, Palau, Micronesia (POIRIER & SMITH, 1974), and another is the population brought to Mauritius in the early 17th century (SUSSMAN & TATTERSALL, 1981). Mauritius is an island which is located 800 km to the east of Madagascar in the southwest Indian Ocean. Generally, an isolated population which has survived for generations on a small island exhibits some isolation-effects such as genetic purification (KAWAMOTO et al., 1988). In primates, it takes a long time for such a phenomenon to become apparent because of their long maturation period and small litter size. Nevertheless, the monkeys on Angaur, whose colonization extends for only 80 years, do show some isolation-effects such as miniaturization of body size and simplification of infestational states by intestinal parasites (MATSUBAYASHI et al., 1989). It can be expected

*This study was carried out under the humane conditions prescribed in "The Guidelines for the Study of Wild Primates and Use of Wild-born Primates" by the Primate Research Institute, Kyoto University, Japan.

that long-term isolation on an island affords monkeys with suitable characteristics as experimental animals such as an increase in genetic uniformity and a decrease of pathogens. The colony of crab-eating macaques in Mauritius, which has occupied the island for nearly 400 years, is considered as a candidate for founding or establishing a new line of biomedical experimental animals. Results of clinical examinations on monkeys in Mauritius have been reported by TATTERSALL et al. (1981) and by IDA et al. (1988), although the numbers of specimens involved were not great. HOUGHTON (1989) reported the possession of antibodies in Mauritius monkeys that were free from herpes B, measles, SRV-1, SRV-2, and STLV-1. To obtain more detailed information on the hematological and serum biochemical values, and natural infections by various pathogenic agents, the present field investigations were carried out in Mauritius in October 1989.

MATERIALS AND METHODS

Animals: The monkeys employed in this study were captured mainly in the south and south-west area of the island of Mauritius (Fig. 1). Trapping was carried out using large net traps set in deer farms. The trapped monkeys were carried by car to Bioculture Mauritius, a breeding farm on the island, and samples were taken from them during the next morning individually under anesthesia by intramuscular injection of ketamine hydrochloride (10 mg/kg). The number of animals examined was 119 in total including juveniles and adults of both sexes but excluding infants.

Hematological and plasma biochemical examinations: These were performed within a few hours after sampling, and the remaining part of the blood samples was centrifuged at

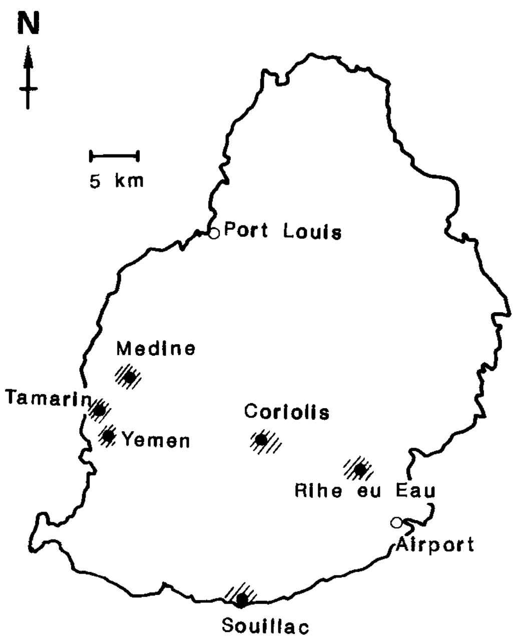

Fig. 1. Locations of trapping areas in Mauritius.

Table 1. Sources of virus antigens used.

Virus	Source
Measles virus	Department of Measles Virus, N. I. H., Tokyo, Japan
SV5	Denka Seiken Co. Ltd., Tokyo, Japan
Herpes simplex virus type 1	Denka Seiken Co. Ltd., Tokyo, Japan
SA6	S. S. KALTER, Virus Reference Laboratory, South Texas Medical Center
Human T cell lymphotropic virus type I	HINUMA, Y., Institute for Virus Research, Kyoto University
Simian immunodeficiency virus/MAC	R. C. DESROSIERS, New England Regional Primate Research Center, Harvard Medical School

3000 rpm for 15 min. The separated plasma samples were kept in a freezer and were brought to Japan in a cooler box filled with dry ice. The RBC count and hemoglobin concentration were measured with a portable spectrophotometer (Compur M 1000, Ames) and the hematocrit value estimated with a portable microcentrifuger (Compur M 1100, Ames). WBC were counted with a Thoma-Zeiss count plate under a microscope. The total plasma protein was determined with a refractometer and the plasma glucose density was measured with a spectrophotometer using the enzyme (GOD) method.

Bacteriological examinations: Parts of the fresh fecal specimens collected from 53 captured animals were transported to the Primate Research Institute, Kyoto University, in screw-capped tubes containing Cary-Blair transport medium for isolation of *Shigella* and *Salmonella* using SS agar plates.

Parasitological examinations: The remainder of the fecal specimens was fixed in 10% Ringer's formalin for parasitic examination using the formalin-ether concentration technique. The sediments of each sample were observed microscopically for the presence of helminth ova and protozoa.

Detection of antibodies to various viruses: The collected plasma was tested for antibodies to SV5, measles virus (MV), herpes simplex virus type 1 (HSV-1), SA6 (cytomegalovirus isolated from the vervet monkey), simian T cell lymphotropic virus type I (STLV-1), and simian immunodeficiency virus/MAC (SIV/MAC). The sources of these virus antigens are listed in Table 1. Antibody to STLV-I was detected by the use of human T cell lymphotropic virus type I (HTLV-I) antigenes, since sequence analysis of the STLV-I genome revealed 90% homology with HTLV-I in the *env. pX* and LTR regions (WATANABE et al., 1985). Hemagglutination inhibition tests for SV5 and MV antibodies were performed by the methods of CHANOCK (1979) with minor modifications and of SHISHIDO et al. (1973), respectively. Complement fixation tests for HSV-1 were carried out by a slightly modified version (INOUE, 1973) of the CDC's standardized method (CENTERS FOR DISEASE CONTROL, 1969). Antibodies to SA6, STLV-I, and SIV/MAC were detected by the indirect immunofluorescence method. Each test was performed according to the techniques of KOBAYASHI (1988), HINUMA et al. (1982) and HONJO et al. (1990), respectively.

Urine examinations: Uric protein and uric sugar were checked by the testape method (Uro-paper, UAG-3, Eiken Chemistry, Tokyo, Japan) using fresh urine that was collected at the time of sampling.

RESULTS

HEMATOLOGICAL AND PLASMA BIOCHEMICAL VALUES

The values for RBC, WBC, Ht, Hb, Tp, and Glu are shown in Table 2. The values of all items measured were within the ranges of normal values reported for Southeast Asian crab-eating monkeys (VERLANGIERI et al., 1985; SUGIMOTO et al., 1986; TAKENAKA, 1986). The data obtained indicate that all individuals were in a good health condition.

URINE EXAMINATIONS

All 13 urine samples which could be collected freshly were negative for protein and sugar.

ISOLATION OF *Shigella* AND *Salmonella*

Shigella and *Salmonella* were not isolated from any monkey.

PARASITOLOGICAL EXAMINATIONS

The results of parasitic inspection of the stools are summarized in Table 3. Three species of protozoans and two species of nematodes were detected in the feces. Of the 53 samples examined, 44 (83%) were positive for one or more species of intestinal parasites.

VIRAL ANTIBODIES

The incidence of antibodies to measles, HSV-1, SA-6, STLV-I, SIV/MAC, and SV-5 is shown in Table 4. All the monkeys examined were negative for measles, HSV-1, SIV/MAC,

Table 2. Hematological values, plasma concentrations of total protein and glucose (mean ± SD) in Mauritius monkeys.

Sex	n	RBC ($10^4/mm^3$)	Ht (%)	Hb (g/dl)	WBC ($10^3/mm^3$)	Tp (g/dl)	Glu (mg/dl)
Male	49	423 ± 72	36.9 ± 3.8	10.9 ± 1.0	8.69 ± 2.58	6.9 ± 0.5	69.0 ± 15.5
Female	70	403 ± 100	36.7 ± 4.0	10.9 ± 1.0	8.14 ± 2.85	6.7 ± 0.6	74.8 ± 18.6

Table 3. Prevalence of intestinal parasites in Mauritius monkeys.

Species	Infestation rate*
Entamoeba coli	28/53 (52.8)
Iodamoeba buetschlii	12/53 (22.6)
Balantidium coli	3/53 (5.6)
Strongyloides fuelleborni	9/53 (16.9)
Trichuris trichiura	9/53 (16.9)
None found	9/53 (16.9)

*Number of positive monkeys/number of monkeys examined (%).

Table 4. Incidence of antibodies to various viruses in Mauritius monkeys.*

Measles	HSV-1	SA-6	STLV-I	SIV/MAC	SV5
0/102 (0)	0/102 (0)	97/102 (95.1)	15/102 (14.7)	0/102 (0)	0/85 (0)

*Number of positive monkeys/number of monkeys examined (%).

and SV-5. Antibody to SA-6 was positive in almost all individuals (95%), but that to STLV-I was positive in only a few individuals (15%).

DISCUSSION

In the evaluation of non-human primates as experimental animals for biomedical research, genetic purification and pathological cleanness are two important factors. Since the genetic characteristics of the Mauritian monkeys will be described in another report, the results of the clinical examinations made on the same population will be discussed here.

Wild crab-eating macaques exported from their original habitat, Southeast Asia, frequently have several kinds of micropathogenes such as *Shigella*, *Salmonella*, pathogenic protozoa, and intestinal parasites (ARYA et al., 1973; CARAWAY et al., 1968; TAKASAKA et al., 1964, 1973; TOKURA & MATSUBAYASHI, 1973; MATSUBAYASHI & SAJUTHI, 1981). On the other hand, it is generally found that a population isolated on a remote island displays a decrease in such micro-organisms and parasites (MATSUBAYASHI et al., 1989).

From this standpoint, it is of interest to know whether or not clean clinical conditions have already been acquired in the population on Mauritius which has a long history of isolation. Hematological examinations yielded normal mean levels for all parameters. TATTERSALL et al. (1981) reported abnormal data for the blood urea, glucose, and triglycerides in three Mauritian monkeys, suggesting the possibility of diabetes mellitus by taking a high carbohydrate diet (sugar cane). However, in the present study, the plasma glucose was in the range of normal levels and sugar was not detected in 13 tested specimens of fresh urine. Nevertheless, in the case of diabetes, it is known that excretion of sugar into the urine varies with time lapse after food intake, and no definitive conclusions can be drawn without undertaking glucose tolerance tests. There have been many reports on the presence of diabetes in non-human primates (DIGIACOMO et al., 1971; JONES, 1974; KIRK et al., 1972; TANAKA et al., 1986) and on abnormal glucose tolerance curves without any clinical signs (HONJO et al., 1976). In the population of Mauritian macaques, therefore, it still remains uncertain whether some individuals may have a genetic predisposition to glucose intolerance or not.

The finding that antibodies to all viruses except SA-6 and STLV-I were negative was in agreement with the reports of HOUGHTON (1989) and IDA et al. (1988). Combining the three sets of results together, the Mauritian monkeys can be said to be free from infection by nine viruses (herpes B, measles, SRV-1, SRV-2, STLV-I, STLV-III, SV5, SV6, and HSV-1). These data indicate that they are clean, particularly on the virological side. However, the results do differ from previous reports on imported crab-eating monkeys from Indonesia, Malaysia, and the Philippines which demonstrated high positivity for many species of viral antibodies (KAWAI et al., 1968; SUZUKI, 1984). The Mauritius macaques are potentially of value as experimental materials, especially in the assay of vaccines for these viruses.

The most frequently detected intestinal parasite was *Entamoeba coli*, being present in 52.8% of the fecal samples, followed by *Iodamoeba buetschlii* (22.6%). Regarding helminths, *Strongyloides fuelleborni* and *Trichuris trichiura* were detected in nine animals each. Two of the animals had multiple infections. Both these species are common parasites which are found frequently in macaque monkeys. Whereas many genera of helminths have generally been detected in crab-eating monkeys (HASHIMOTO & HONJO, 1966; HONJO et al., 1963; KOYAMA et al., 1976; MATSUBAYASHI & SAJUTHI, 1981; WONG & CONRAD, 1978), the variety of helminth species found in the Mauritius monkeys was clearly relatively small. Similar results have been obtained for the crab-eating monkeys on Angaur Island

(MATSUBAYASHI et al., 1989) and rhesus monkeys on the island of Cayo Santiago (KESSLER et al., 1984).

These findings indicate that the helminth fauna in a population on an isolated island tends to be relatively simple compared to that in a population occupying continental or broad environments. Intestinal helminth infection depends largely on the climate prevailing in the areas where the animals live. For instance, it was reported that only *Trichuris trichiura* was detected in Japanese monkeys living in a snowy area (NIGI et al., 1975).

It seems likely that a small population of monkeys introduced to an island may accidentally harbor a few intestinal helminths, and/or that the helminth eggs or larvae cannot survive or continue to infect because of a lack of intermediate hosts. Such simplification of viral infection and parasitic infestation may represent a general trend in populations arising through isolation on a small island for considerable periods. There appear to be two steps in the development of such a decrease of pathogens in insulated populations. One involves the number of individuals taken from the mother population. When the number of individuals is small at the time of first being transplanted from the original habitat, the number of pathogenic species may not be so great as that in the whole ancestral population. The second step concerns the problem of adaptation of pathogens to unfamiliar environments. For example, part of the life cycle of parasites such as infestation of intermediate hosts may sometimes be cut under the new colonizing circumstances. At least, the simplification of the viral and parasitic fauna of the Mauritius macaques can be said to be characteristic when compared to the same species living in Southeast Asia, and also to the rhesus monkeys in China.

It is difficult to judge whether or not the Mauritius monkeys have been subject to any genetic deterioration through breeding as a closed colony from a small number of founders for a period of nearly 400 years. In Mauritius, four monkeys that have an impediment of their motor nerves are kept in captivity, and there is also personal information that the frequency of disorders of the retina is much higher in Mauritian macaques than in Indonesian ones. It is unclear whether these symptoms are based on genetic factors or on Physiological factors. Even if they do have a genetic background, however, such genetic deterioration by inbreeding depression appears to be very rare in this species.

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Authors' Names and Addresses: KIYOAKI MATSUBAYASHI, SHUNJI GOTOH, YOSHI KAWAMOTO, *Primate Research Institute, Kyoto University, Inuyama, Aichi, 484 Japan*; TSUYOSHI WATANABE, *Sugiyama Jyogakuen University, Nisshin-cho, Aichi-gun, 470-01 Japan*; KEN NOZAWA, *Chukyo University, Toyota, Aichi, 470-03 Japan*; MASAO TAKASAKA and TOYOKO NARITA, *Tsukuba Primate Center for Medical Science, Tsukuba, Ibaraki, 305 Japan*; OWEN GRIFFITHS and MARY-ANN STANLEY, *Bioculture (Mtius) Ltd., Mauritius.*