

SEATO Medical Research Study on Laboratory Animals.

coordinator: John H. morris, LtCol, VC Chief, Dept of Veterinary Medicine

Principal Investigators: Norman C. Negus, PhD*
Richard O. Spertzel, Capt, VC

Associate Investigators: Robert L. Taylor, LtCol, MSC
Billy W. Evans, Major, MSC

Assistant Investigators: Prayot Tanticharoenyos, D.V.M.
Verachart Chaicumpa, D.V.M.
Kwanyuen Lawhaswasdi, D.V.M.

Period of Report: 1 April 1965-31 March 1966

General Information

Investigations relative to colonization of the tree shrew (Tupaia glis) have resulted in establishment of a nucleus breeding colony. Hopefully this will provide a small prosimian primate for research purposes. Further investigation of its value as an experimental animal is required.

The gibbon (Hylobates lar lar) is an excellent laboratory primate for research investigations. Colonization of this species must however be considered as a long-term program. Further investigation in methods of caging, nutrition and all aspects of maintenance is required. Construction of holding cages approximately 100 square feet in area is under consideration for investigation of monogamous mating.

Animals produced (mice, rats, guinea pigs and hamsters) are available in sufficient numbers to meet planned or anticipated research requirements.

*Delta Primate Center, Tulane University.

STUDY REPORTS

1. Title: "Ecology of the Gibbon (Hylobates lar lar)"

Principal Investigators: John H. Morris, LtCol, VC
Norman C. Negus, PhD*
Richard O. Spertzel, Capt, VC

Associate Investigators: Robert L. Taylor, LtCol, MSC
Billy W. Evans, Major MSC

Assistant Investigators: Cecil Pugh, Jr., SSG (E-6)
Charlie Horton, SFC (E-7) P1
Charles J. Stouffer, SSG (E-6)
Eugene F. Clary, SFC (E-7)
James R. Crook, Capt, MSC
Philip Z. Sobocinski, Capt, MSC
Prayot Tanticharoenyos, D.V.M.
Verachart chaicumpa, D.V.M.

OBJECTIVE: The objective of this study is to colonize this species under laboratory conditions, evaluate its potential as a laboratory animal and to determine biological norms.

DESCRIPTION: Three species of gibbon are found in Thailand. These are Hylobates lar lar, Hylobates pileatus and Hylobates conicolor. Since the lar lar or "white handed" gibbon is the most common, efforts to colonize and obtain biological data have been limited to these. These animals have not been utilized as laboratory animals in the past and very little data have been published. Data available at present are limited to behavioral patterns of the species in the natural habitat and limited reports of reproduction in zoos. Since this species is one of the ape family and rather high on the evolutionary tree, it would seem to be an ideal laboratory animal because of its docility and susceptibility to certain infections. This program had been aimed at colonizing the species in laboratory and semi-zoo environments and evaluating its potential as a laboratory animal.

PROGRESS: During the year, fifty-five juvenile gibbons 2-3 years of age were purchased to evaluate the species as an experimental host for malaria. Twenty-five male and female animals 4-6 years of age have been procured as future breeders. The juvenile animals are maintained in individual cages either 2' wide 4' high and 4' deep or 2' wide 3' high and 4' deep. All cages are equipped with a stainless steel perch across one end and an exercise bar located at the midpoint near the top. The smaller cages can be stacked two high, to conserve space. Animals purchased as breeders are placed in large cages of 600 or 200 square feet. Four males and six females have been held in the larger cage and although fighting has occurred few serious injuries have developed. One male and two females may be maintained in the smaller cage and no fighting has been observed. Day-long observations were made to collect

* Delta Primate Center, Tulane University

data on compatibility, activity and mating behavior. The general activity pattern followed that observed in natural populations. The gibbons became active shortly before daylight and engaged in considerable feeding activity during the early morning hours. Considerable vocalization also occurred during this period. Much grooming, mock fighting and vocal response to human visitors occurred during most of the day, with a marked resting period from the late morning into the early afternoon. Activity again increased in the late afternoon prior to sunset.

Following procurement, all animals are quarantined at Bangkok for approximately 30 days. During this period they are injected intrapalpebrally with tuberculin diluted 1:10 and read at 24, 48 and 72 hours. Three animals have had positive reactions. Thin and thick blood smears were made to determine presence of malarial parasites and to date all have been negative. Fecal specimens are obtained on individual animals for parasitologic and bacteriologic examination. When indicated, animals are treated. Samples of blood for laboratory studies are obtained within 72 hours following introduction into the quarantine area. After quarantine all animals are transported to Prabuddahbat.

Hematology, blood type, biochemical and electrophoretic data are summarized in the following tables:-

TABLE I
HEMATOLOGY
71 Gibbons

Value	Average	Range
Hct %	41	29-52
Hgb (gm%)	12.0	7.0-16.4
WBC x 1000	12.0	3.4-38.5
RBC x 10 ⁶	6.57	3.48-8.54
N %	49	20-80
L %	43	8-76
B %	2.3	0-17
E %	0-1	0-4
Baso %	1-2	0-5
M %	3-4	0-8

Blood Type

A	B	AB	O
8	15	13	0

Lymphocyte counts increase markedly following splenectomy while neutrophils decreased. Leucocyte counts appear to more nearly approach those recorded for other animals than do those of the tree shrew (Tupaia glis).

TABLE II
BIOCHEMISTRY

	Average	Range	# Animals
BUN	12.6 ⁺ _{5.3}	2.5-28.8	74
glucose	90 ⁺ ₂₆	35-190	59
cholesterol	155 ⁺ ₄₁	81-266	68
Bilirubin total	0.2 [±] _{0.1}	0-5	68
Thymol Turbidity	1.2 ⁺ _{.4}	0.0-2.9	29
Alkaline Phosphatase	20.8 ⁺ _{13.1}	3.0-72.0	56
Acid Phosphatase	0.8 ⁺ _{0.34}	0.20-1.53	39
SGOT	34 ⁺ ₁₅	14-96	71
SGPT	26 ⁺ ₈	9-49	71
CO ₂	13.3 ⁺ _{5.3}	3.5-25.8	70
Cl ⁻	106 ⁺ ₅	89-125	69
Na ⁺	147 ⁺ ₅	130-159	70
K ⁺	5.2 [±] _{1.1}	3.3-7.8	70
Ca ⁺⁺	10.0 ⁺ _{0.8}	8.6-12.2	60
PO ₄ [≡]	5.2 ⁺ _{1.4}	2.4-8.5	58
Uric Acid	2.5 ⁺ _{1.0}	0.3-8.2	44

TABLE III
ELECTROPHORESIS VALUES

	Average	Range
Total Protein	6.5 $\frac{+}{-}$ 0.7	4.8-8.3
alpha ₁	0.1	*0.0-0.3
alpha ₂	1.0 $\frac{+}{-}$ 0.45	0.3-2.8
beta	0.8 $\frac{+}{-}$ 0.3	0.4-1.9
gamma	1.3 $\frac{+}{-}$ 0.5	0.4-2.7
albumin	3.4 $\frac{+}{-}$ 0.8	1.2-4.9
A/G ratio of means	N.A	N.A 1.10

* Only two animals had a detectable alpha₁ globulin.

The values in Tables I, II & III are on newly procured animals. Since these animals have been subjected to various stresses (e.g. shipping, changing diet, etc.) and probably less than adequate diet, these values tend to show a great deal of variability. It is anticipated that after 6 months or longer, animals not under experimental use will be checked again and results will provide better estimates of normal values. The absence of an alpha₁ globulin in gibbons is worth noting. A similar observation has been made in a few M. iris monkeys but not in M. speciosa. Difference between sexes, age or colonized groups as reported for the tree shrews has not been observed with the gibbons. An interesting finding was that although red blood cells in gibbons with a normal (40 to 45) Hct but a very low Hg (10. to 12 gm%), would be expected to be hypochromic, they do not appear to be so. Whether or not a hemoglobin exists which does not react with the methemoglobin cyano hemoglobin test remains unanswered.

Fecal specimens obtained from individual animals were sent to the bacteriology and parasitology laboratories at SMRL. Strongyloides, trichuris, roundworm, and hookworm were observed in approximately 50% of the animals examined. E. coli, E. nana and Giardia cysts were found in limited numbers and Taenia ova were found in a specimen obtained from one individual.

Dizan powder administered orally has been found to be quite effective in the elimination of these endoparasites. The usual bacterial flora was isolated. Three animals shedding shigella sp. were treated with antibiotics. Approximately 10% of newly procured gibbons are carrying one or more enteric pathogens. The following pathogens have been isolated.

Pathogenic	<u>E. coli</u>	O125:B15
	<u>E. coli</u>	O112:B11
	Shigella flexnerii	6
	Shigella dysenteriae	1
	Shigella sonnei	form 1
	Shigella flexnerii	3

Investigation of skin lesions resulted in the isolation of an unusual dermatophytic fungus. The organism grossly and microscopically has many of the characteristics of the genus *Microsporum*, but is sufficiently atypical to preclude speciation without additional study. Subcultures of the organism have been sent to the Communicable Disease Center, Atlanta, Georgia where single spore matings can be performed to determine whether this is an atypical strain of one of the recognized species of *Microsporum* or a new species. Twenty two gibbons have been cultured and the organism recovered from nineteen.

In early January, an upper respiratory illness broke out and spread rapidly through the colony. The onset started a week to ten days after one of the caretakers was ill with a severe URI. The disease followed the usual pattern of a highly contagious airborne infection in a susceptible population (i.e. one or two isolated cases and then a rapidly spreading illness), involving all thirty animals in the colony with a few animals developing prolonged infection with lower respiratory tract involvement and two deaths. A virus (s) was isolated but is not identified yet. Duration of epidemic was about 5 weeks.

SUMMARY: Investigations in caging indicate that this species requires increased space over that required for most other primates. Exercise is essential and is primarily brachiation. Equipment must, therefore, be provided in cages where they are to be held for extended periods. Mating has been observed in the caged animals but pregnancy has not been substantiated. Hematologic data are comparable to that of most other primates, however the absence of alpha₁ globulin is worth noting. Approximately 50% of the animals procured are infested with one or more endoparasites and pathogenic (*E. coli* and *Shigella* sp.) have been recovered from a significant number. Dermatitis occurs commonly and is thought to be due to a species of *Microsporum*. Animals may be quite susceptible to upper respiratory infections. Splenectomized juveniles are very susceptible to *P. falciparum* infection. (See Studies on Malaria)