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# HEALTH EVALUATION OF FREE-RANGING AND SEMI-CAPTIVE ORANGUTANS (*PONGO PYGMAEUS PYGMAEUS*) IN SABAH, MALAYSIA

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**ABSTRACT:** Baseline data on health of free-ranging wildlife is essential to evaluate impacts of habitat transformation and wildlife translocation, rehabilitation, and reintroduction programs. Health information on many species, especially great apes, is extremely limited. Between 1996 and 1998, 84 free-ranging orangutans captured for translocation, underwent a complete health evaluation. Analogous data were gathered from 60 semi-captive orangutans in Malaysia. Baseline hematology and serology; vitamin, mineral and pesticide levels; and results of health evaluations, including physical examination, provide a baseline for future monitoring. Free-ranging and semi-captive orangutans shared exposure to 11 of 47 viruses. The semi-captive orangutans had significantly higher prevalence of antibodies to adenovirus ( $P < 0.0005$ ) and rota (SA 11) virus ( $P < 0.008$ ). More free-ranging than semi-captive animals had antibodies to Japanese encephalitis virus ( $P < 0.08$ ) and foamy virus ( $P = 0.05$ ). Exposure to parainfluenza and langat viruses was detected exclusively in semi-captive animals and exposure to sinbis virus was only found in free-ranging orangutans. There was evidence of exposure to respiratory syncytial virus, coxsackie virus, dengue virus, and zika virus in both groups. Ebstein-Barr virus was ubiquitous in both groups. Prevalence of antibodies against mumps virus changed from 0% in 1996 to 45% in 1998. No antibodies were detected to many important zoonotic viral pathogens, including herpesvirus and hepatitis virus. Prevalence of *Balantidium coli* and *Plasmodium pitheci* infections and exposure to mycobacterium was higher in the semi-captive animals. Differences in exposure to pathogens between the groups may be due to environmental factors including differences in exposures to other species, habitat quality, nutritional status, and other potential stressors. Differences in health parameters between captive and free-ranging orangutans need to be considered when planning conservation areas, translocation procedures, and rehabilitation protocols. Because survival of the orangutan is linked to animal and ecosystem health, results of this study will assist wildlife conservation programs by providing baseline health information.

**Key words:** Adenovirus, arbovirus, conservation, demographics, orangutan, *Plasmodium*, rotavirus, wildlife, zoonosis.

## INTRODUCTION

Two subspecies of orangutan, *Pongo pygmaeus abelii* and *P. pygmaeus pygmaeus* (Zhi et al., 1996) are found in Indonesia and Malaysia and population estimates may be as low as 10,000 individuals (Andau, 1994). These arboreal great apes exist naturally at low population densities, approximately two individuals per km<sup>2</sup> (Wolfheim, 1983). Males are generally solitary, while females often travel with an infant and/or juvenile offspring, although group size can fluctuate in relation to fruit availability. Orangutans are currently listed

as endangered and their populations continue to decline due to loss of habitat, human population expansion, and illegal capture and trade (Robertson and van Schaik, 2001). Disease, possibly linked to environmental changes, may play a role by affecting mortality, morbidity and fecundity. Infectious and noninfectious diseases may shape wildlife behavior and ecology (Dobson and Hudson, 1986; Colborn et al., 1997). The risks of over-crowding, inappropriate habitat, unsafe water, and waste disposal for transmission of water-borne and respiratory infections are well recognized (Tsai, 1994) as well as predisposing

individuals to the transmission of vector-borne and zoonotic infections. Great apes face threats caused by environmental change and pathogens shared between non-human primates and humans (Kalter et al., 1967).

The objectives of this study were to obtain 1) baseline health information on free-ranging orangutans and 2) health data for development of protocols for rehabilitation and release programs.

## MATERIALS AND METHODS

Between 1996 and 1998, free-ranging (FR) orangutans were translocated as part of a conservation project conducted by the Sabah Wildlife Department (SWD; Sabah, Malaysia). Translocation of these primates from small isolated patches of forest due to be logged to protected habitat provided SWD, assisted by the Wildlife Conservation Society (WCS; Bronx, New York, USA), the opportunity to conduct thorough health evaluations on these animals. To compliment the FR orangutan health evaluations, similar protocols were used to evaluate semi-captive (SC) orangutans housed at the Sepilok Orangutan Rehabilitation Center (SOURC; Sandakan, Malaysia).

### Site description

Free-ranging orangutans were evaluated during translocation from forest fragments or degraded habitat in Eastern Sabah (Malaysia) to the protected Tabin Wildlife Reserves (TWR; 05°12.692'N, 118°38.738'E) south of the Segama River (Sabah, Malaysia). All capture sites were within 200 km of the TWR and included 15 sites of primary and secondary rain-forest patches >200 ha surrounded by landscapes cleared for agriculture, devoid of trees, or replaced with oil palm plantations. The TWR consists of 1,600 km<sup>2</sup> of protected primary and secondary lowland tropical rain forest. Historic and resident population densities of orangutans are low (Andau et al., 1994).

Semi-captive confiscated, orphaned, or injured orangutans undergoing rehabilitation were housed in cages and/or released daily at SOURC (05°51.841'N, 117°57.003'E). This orangutan rehabilitation center is located on the northern edge of the Sepilok Forest Reserve, a 400-km<sup>2</sup> reserve of protected primary and secondary forest outside Sandakan, Sabah. Both sites are under SWD management.

### Populations and study period

Between August 1996 and May 1998, FR orangutans were immobilized, examined, transported to the TWR, and released within 48 hr of capture. Orangutans were divided into four age classes based on weight and dental morphology. Age groups were: infant <1 yr; juvenile 1–5 yr old; subadult 6–10 yr old; and adult >10 yr of age. Orangutans were identified by tattoo and transponder chip (Trovan®, Electronic Identification Devices, Santa Barbara, California, USA), freeze branding, and photographs.

Over 130 orangutans resided at SOURC during this study; most were from Sabah. Samples were evaluated from 60 of these SC individuals. After a 60-day quarantine, orangutans progressed from cages to a SC status in Sepilok Forest Reserve where they are fed bananas and milk daily. Animals were classified into age classes as for FR orangutans.

### Sample and data collection

Most FR animals were immobilized with either tiletamine-zolazepam hydrochloride (Telazol®, 100 mg/ml, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa, USA; *n*=66) or ketamine (Ketaset®, Fort Dodge Laboratories, Inc.) and xylazine (Rompun, Miles Agricultural Division, Animals Health Products, Mission, Kansas, USA; *n*=18). When required, juveniles and infants were manually restrained. Based on visual weight estimates, dosages of 7 mg/kg of ketamine and 1.4 mg/kg xylazine or 3 mg/kg of Telazol® were chosen. Drugs were administered intramuscularly, via rifle or pistol with 3 ml darts (Telinject® USA, Saugus, California; Kilbourn et al., 1997). If the SC animals required immobilization, 3 mg/kg dose of Telazol® was given intramuscularly by hand.

Following immobilization, physical examinations were performed, and 2–15 ml of blood were collected from cephalic, saphenous, or femoral veins. Blood was placed in plain, ethylenediaminetetraacetic acid, heparinized, and non-vulcanized rubber top tubes immediately. Plasma and serum were harvested within minutes after centrifugation and aliquots placed into vials (cryovials®, Nalge Company, Rochester, New York) and frozen in liquid nitrogen. Blood cells were processed with glycerolite prior to freezing according to the techniques described by Cox-Singh et al. (1997) for subsequent detection of *Plasmodium* spp. Thick and thin peripheral blood films (PBF) were made. Thin smears were dried and fixed in methanol (Diff-quick®, Baxter Healthcare Corp., Miami, Florida, USA) for differential counts. Thick films were allowed to dry and then the cells

were lysed in an aqueous solution. Three spots of approximately 70  $\mu$ l of whole blood were placed onto Whatman 3MM<sup>®</sup> (Minnesota Mining and Manufacturing Co., St. Paul, Minnesota, USA) chromatography paper, dried, and placed with desiccant packages of silica beads into plastic bags. For the Knott's technique, a concentration procedure using blood and dilute formalin designed to detect microfilaria, a small volume of blood (<1 ml) was mixed with 11 ml 2% formalin. Hairs, using gloves or sterile forceps, were plucked and placed in paper envelopes for genetic analysis. Fecal samples were placed in polyvinyl alcohol (PVA<sup>®</sup> Para-pak, Meridian Diagnostics Inc., Cincinnati, Ohio, USA) and/or 10% phosphate buffered formalin and wide-mouth plastic vials.

### Sample analysis

Hematocrit, total solids, and white blood cell (WBC) counts using the Unopette<sup>®</sup> system (Beckton Dickinson & Co., Franklin Lakes, New Jersey, USA), were conducted in the field. Dif-quick<sup>®</sup> stained samples were used for WBC differentials, performed at the SOURC laboratory. Serum was processed on an automated analyzer (Ciba Corning Alliance 580 Auto Analyzer, Ciba Corning Diagnostics Corp., New York, New York) at a commercial veterinary laboratory (ANTECH Diagnostics, New York, New York). Serum mineral analysis was conducted at Michigan State Animal Health Diagnostic Laboratory (MSHDL, East Lansing, Michigan, USA) by inductively coupled argon plasma emission spectroscopy as described by Stowe et al. (1993). Plasma samples were analyzed at the WCS Nutrition Laboratory for alpha-tocopherol, beta-tocopherol, and retinol as described by Dierenfeld et al. (1993). Chlorinated and polychlorinated biphenyl pesticides (aldrin; alpha-BHC; beta-BHC; O, P'-DDD; P, P'-DDD; P, P'-DDE; O, P'-DDT; P, P'-DDT; dieldrin; endrin; heptachlor epoxide; lindane (gamma-BHC); and nonachlor) were analyzed at MSHDL using the method described by Price et al. (1986).

Thirty-nine non-arthropod borne and eight arthropod-borne viruses (Tables 1, 2) were chosen based on their potential to infect both human and/or non-human primates (Heberling and Kalter, 1986; Marchette, 1994; Kalter et al., 1997). Serologic techniques described by Kalter and Heberling (1971) and Kalter et al. (1997) were conducted at the Virus Reference Laboratory (San Antonio, Texas, USA). Analyses for vector-borne viral diseases were conducted by the Centers for Disease Control and Prevention (Arbovirus Branch, Atlanta, Georgia, USA), and Harvard School of Public

Health (Boston, Massachusetts, USA) and selected based on potential agents in the region (Tesh et al., 1975; Wolfe et al., 2001). Analyses for flavivirus antibodies used enzyme-linked immunosorbent assays and immunofluorescent antibody testing. Antibody titers of 1:20 were considered positive of exposure to the virus of interest or a closely related strain (Wolfe et al., 2001). Ninety-eight serum samples, 54 from FR and 44 SC orangutans were tested for antibodies to viruses listed in Tables 1 and 2. Testing for foamy viruses was only conducted on 1998 samples and the sample size was smaller.

Detection of antigen 85 (Ag85), a protein produced by actively reproducing mycobacteria, was used as an indicator of active mycobacterial infection in FR animals (Kilbourn et al., 2001). Intradermal skin testing and Ag85 analysis were done on SC animals. Animals were tested for antibodies to 17 serovars of *Leptospira interrogans* by microscopic agglutination test conducted at the New York State Veterinary Diagnostic Laboratory (Cornell University, Ithaca, New York; positive if  $\geq 1:100$ ). Bacteria were isolated from fecal samples, lesions, and/or organs in a few SC animals on routine culture and sensitivity testing. Cultures were not run on FR animals due to logistical constraints.

A total of 84 orangutans were examined for *Plasmodium* spp. Of these, 45 samples were from FR orangutans and 39 were from SC orangutans at SOURC. Polymerase chain reaction (PCR) assays and microscopic examination of Giemsa-stained peripheral blood films were used. These films were screened under low and high power for *Plasmodium*, either by laboratory technologists at the district hospital or by officers from the Sabah State Vector-Borne Disease Control Office (Sandakan, Sabah, Malaysia). Blood spots on filter paper were extracted using InstaGene<sup>®</sup> extraction kit (Bio-Rad Laboratories, Hercules, California) as described (Cox-Singh et al., 1997; Wolfe et al., 2001). Extracted DNA was subject to a *Plasmodium* genus-specific PCR assay modified from a nested PCR protocol (Singh et al., 1996). Fecal samples were analyzed for gastrointestinal parasites by direct smears and flotation followed by microscopic evaluation. A subjective estimations of parasite loads were made based on the number of parasites seen per low power field (low, <1; moderate, 1–5; and high, >5 parasites per low power field).

Results were analyzed using a Chi-square test and Fisher's exact test (Hennekens and Buring, 1987) and Epitool Statistical Application (Carolyn Masters-Williams; Epitool v. 1.03, John Hopkins Hospital, Department of Health-

TABLE 1. Results of viral serology in orangutan from Sabah, Malaysia.

Virus	Semi-captive		Free-ranging		Test
	Number positive/ number tested (%)	Number positive/ number tested (%)	Number positive/ number tested (%)	Number positive/ number tested (%)	
<b>Non-arboviruses</b>					
Herpes virus simiae B <sup>a</sup>	0/44 (0%)	0/54 (0%)	0/44 (0%)	0/54 (0%)	a
Chimpanzee cytomegalovirus <sup>a</sup>	0/44 (0%)	0/44 (0%)	0/44 (0%)	0/54 (0%)	a
Coxsackie B <sup>a</sup>	0/44 (0%)	0/44 (0%)	0/44 (0%)	0/54 (0%)	a
Coxsackie B-1 <sup>a</sup>	0/44 (0%)	0/44 (0%)	0/44 (0%)	0/54 (0%)	a
Coxsackie B-2 <sup>a</sup>	0/44 (0%)	0/44 (0%)	0/44 (0%)	0/54 (0%)	a
Coxsackie B-3 <sup>a</sup>	0/44 (0%)	0/44 (0%)	0/44 (0%)	0/54 (0%)	a
Coxsackie B-5 <sup>a</sup>	0/44 (0%)	0/44 (0%)	0/44 (0%)	0/54 (0%)	a
Coxsackie B-6 <sup>a</sup>	0/44 (0%)	0/44 (0%)	0/44 (0%)	0/54 (0%)	a
Encephalomyocarditis <sup>a</sup>	0/44 (0%)	0/44 (0%)	0/44 (0%)	0/54 (0%)	a
Filo-group Ebola-Reston and Marburg <sup>a</sup>	0/44 (0%)	0/44 (0%)	0/44 (0%)	0/54 (0%)	a
Influenza A <sup>a</sup>	0/44 (0%)	0/44 (0%)	0/44 (0%)	0/54 (0%)	a
Influenza B <sup>a</sup>	0/44 (0%)	0/44 (0%)	0/44 (0%)	0/54 (0%)	a
Hepatitis A—total Ag <sup>b</sup>	0/44 (0%)	0/44 (0%)	0/44 (0%)	0/54 (0%)	b
Hepatitis B—total Ag <sup>b</sup>	0/44 (0%)	0/44 (0%)	0/44 (0%)	0/54 (0%)	b
Herpes hominis 1 <sup>a</sup>	0/44 (0%)	0/44 (0%)	0/44 (0%)	0/54 (0%)	a
Herpes hominis 2 <sup>a</sup>	0/44 (0%)	0/44 (0%)	0/44 (0%)	0/54 (0%)	a
Rubeola <sup>a</sup>	0/44 (0%)	0/44 (0%)	0/44 (0%)	0/54 (0%)	a
Monkey pox <sup>a</sup>	0/44 (0%)	0/44 (0%)	0/44 (0%)	0/54 (0%)	a
Parainfluenza 1 <sup>a</sup>	0/44 (0%)	0/44 (0%)	0/44 (0%)	0/54 (0%)	a
Parainfluenza 2 <sup>a</sup>	0/44 (0%)	0/44 (0%)	0/44 (0%)	0/54 (0%)	a
Poliomyelitis <sup>a</sup>	0/44 (0%)	0/44 (0%)	0/44 (0%)	0/54 (0%)	a
Poliomyelitis 1 <sup>a</sup>	0/44 (0%)	0/44 (0%)	0/44 (0%)	0/54 (0%)	a
Poliomyelitis 2 <sup>a</sup>	0/44 (0%)	0/44 (0%)	0/44 (0%)	0/54 (0%)	a
Poliomyelitis 3 <sup>a</sup>	0/44 (0%)	0/44 (0%)	0/44 (0%)	0/54 (0%)	a
Rubella <sup>a</sup>	0/44 (0%)	0/44 (0%)	0/44 (0%)	0/54 (0%)	a
African green monkey herpes <sup>a</sup>	0/44 (0%)	0/44 (0%)	0/44 (0%)	0/54 (0%)	a
African green monkey cytomegalovirus <sup>a</sup>	0/44 (0%)	0/44 (0%)	0/44 (0%)	NA	a
Simian Varicella-Zoster <sup>a</sup>	0/44 (0%)	0/44 (0%)	0/44 (0%)	0/54 (0%)	a
Simian immunodeficiency <sup>a</sup>	0/44 (0%)	0/44 (0%)	0/44 (0%)	0/54 (0%)	a
Simian retrovirus <sup>a</sup>	0/44 (0%)	0/44 (0%)	0/44 (0%)	0/54 (0%)	a
Varicella-zoster <sup>a</sup>	0/44 (0%)	0/44 (0%)	0/44 (0%)	0/54 (0%)	a
Batai <sup>c</sup>	0/31 (0%)	0/31 (0%)	0/31 (0%)	0/40 (0%)	c
Chikungunya <sup>c</sup>	0/31 (0%)	0/31 (0%)	0/31 (0%)	0/40 (0%)	c

<sup>a</sup> Dot-immunobinding assay, Veterinary Reference Laboratory (VRL), Simian Diagnostic Laboratory, San Antonio, Texas, USA.

<sup>b</sup> Enzyme-linked immunoassay (ELISA), VRL, Simian Diagnostic Laboratory.

<sup>c</sup> Immunofluorescence and ELISA, VRL, Simian Diagnostic Laboratory and Centers for Disease Control and Prevention, Atlanta, Georgia, USA.

TABLE 2. Results of viral serology of orangutan from Sabah, Malaysia.

Virus		Semi-captive; Number positive/ number tested (%)	Free-ranging; Number positive/ number tested (%)	Total positive %
Non-arboviruses	Adenovirus group <sup>a</sup>	18/44 (40%)	3/54 (5%)	21
	Coxsackie B-4 <sup>a</sup>	1/44 (2.2%)	1/54 (1.8%)	2
	Ebstein-Barr <sup>b</sup>	44/44 (100%)	54/54 (100%)	100
	Foamy <sup>a</sup>	10/24 (41%) <sup>c</sup>	15/20 (75%) <sup>c</sup>	25
	Mumps <sup>a</sup>	11/44 (25%) <sup>d</sup>	11/54 (20%) <sup>d</sup>	22
	Parainfluenza 3 <sup>a</sup>	1/44 (2.2%)	0/54 (0%)	1
	Respiratory syncytial <sup>a</sup>	2/44 (4.5%)	1/54 (1.8%)	3
	Rotavirus SA11 <sup>a</sup>	25/44 (56%)	15/54 (27%)	40
	Arboviruses	Dengue <sup>b</sup>	10/31 (32%)	11/40 (28%)
Japanese encephalitis <sup>b</sup>		5/31 (16%)	14/40 (38%)	28
Langat <sup>b</sup>		1/31 (3%)	0/40 (0%)	1
Sinbis <sup>b</sup>		0/31 (0%)	4/40 (10%)	5
Tembusu <sup>b</sup>		1/29 (3%)	1/39 (3%)	3
Zika <sup>b</sup>		1/31 (3%)	5/40 (13%)	8

<sup>a</sup> Dot-immunobinding assay at VRL Simian Diagnostic Laboratory.

<sup>b</sup> Immunofluorescent antibody (commercial IFA) and/or ELISA VRL Simian Diagnostic Laboratory and Centers for Disease Control and Prevention.

<sup>c</sup> No samples from 1996 or 1997 were tested for this virus; only 1998 samples were tested.

<sup>d</sup> All samples from 1996 and 1997 were negative (0/50); all positive samples are from 1998 (22/48).

care Epidemiology and Infection Control, Baltimore, Maryland, USA).

## RESULTS

A total of 84 FR orangutans was studied. Sixty-two percent ( $n=52$ ) of FR were adults compared to only 13% ( $n=17$ ) SC animals. The percent of FR females captured for translocation (56%,  $n=47$ ), was similar to the percent of females studies in the SC population (53%,  $n=68$ ). Nine FR females were accompanied by a juvenile. We assumed juveniles with females were offspring. No captured female appeared pregnant upon palpation. Most FR animals appeared healthy but lean. One female had a grade III systolic heart murmur and several animals had superficial abrasions on their hands and feet. One adult female had a laceration on her lower lip. Two FR animals had no significant lameness or dysfunction despite having lesions suggestive of previously healed fractures of humerus and femur. Two adult males had unilateral corneal opacities.

A total of 129 SC orangutans had physical exams. Physical injuries were common

on all adult and a few juvenile animals upon arrival to SOURC. Predominant injuries included fractured bones, and knife, rope, or chain induced injuries. Only one injury, a fractured femur, had not healed at the time blood samples were collected. Most animals had been housed at SOURC for several months to years or were completing or had finished their quarantine. Nasal discharge and loose stool were common findings. Corneal opacities were seen in three adult males.

## Blood and fecal analysis

Serum chemistries, serum soluble elements, and plasma vitamins for FR orangutans are presented in Tables 3–5. Chlorinated pesticides and polychlorinated biphenyls (PCBs) were not detected in samples analyzed.

Viruses for which antibodies were detected are listed in Tables 1 and 2. Exposure to mumps virus in both FR and SC populations changed significantly during the study, with no positive antibody titers in 1996 and 1997 (0/50 combined samples tested prior to 1998) whereas 45% (22/48

TABLE 3. Serum chemistries values from 38 free-ranging orangutan from Sabah, Malaysia.

Serum parameters	Units	Mean	SD	Range
Albumin/globulin ratio		1.56	0.33	0.85–2.18
Albumin	g/dl	4.07	0.55	2.9–5.6
Alkaline phosphatase	IU/l	288.9	186.3	2–1,186
Alanine aminotransferase	IU/l	65.9	88.8	14–520 <sup>a</sup>
Amylase	IU/l	187	565	23–3,538
Aspartate aminotransferase	IU/l	246	490	13–2,844 <sup>a</sup>
Blood urea nitrogen	mg/dl	10.8	13.4	1.0–80
Blood urea nitrogen/creatinine ratio		9.48	7.7	1.0–39
Calcium	mg/dl	8.46	1.32	2–10.8
Calcium/phosphorus ratio		2.55	135.5	1.12–4.5
Cholesterol	mg/dl	161.9	165.9	77–1,125
Chloride	mg/dl	97.5	1.63	11–118
Carbon dioxide	mmol/l	15.2	1.67	14–21
Creatine kinase	IU/l	2497	3767	72–10,000 <sup>a</sup>
Creatinine	mg/dl	1.4	2.2	0.7–11
Gamma glutamyltransferase	IU/l	14.7	10.6	1.0–48
Globulin	gm/dl	2.6	0.42	2–3.5
Glucose	mg/dl	118.7	47.5	57–275
Potassium	meq/l	4.55	97.5	3–8.9
Lactate dehydrogenase	IU/l	1424.8	2507	58–10,000 <sup>a</sup>
Lipase	IU/l	23.2	22.4	2–126
Magnesium	mg/dl	1.63	0.41	0.7–3
Sodium	meq/l	135.5	4.55	34–157
Phosphorus	mg/dl	3.68	2.55	1.9–7.3
Total bilirubin	mg/dl	0.57	0.54	0.1–2.6
Total protein	gm/dl	6.75	0.63	5.5–8.5
Triglyceride	mg/dl	71.2	30.4	27–161

<sup>a</sup> Mean higher than International Species Information System physiologic reference value.

combined samples tested in 1998) of the samples were positive in 1998 ( $P < 0.003$ ). Only SC orangutans had antibodies to parainfluenza 3 (2%) and langkat virus (3%) whereas only FR animals had antibodies to sinbis virus (10%) ( $P = 0.075$ ). Prevalence of antibodies to adenovirus ( $P < 0.003$ ) and rotavirus SA 11 ( $P < 0.003$ ) were significantly higher in the SC than FR animals. Prevalence of antibodies to Japanese encephalitis virus ( $P < 0.08$ ) and foamy virus ( $P = 0.05$ ) was higher in FR orangutans. Prevalence of antibodies to non-arboviral pathogens in the SC orangutans was significantly higher than in FR animals ( $P = 0.01$ , relative risk [RR] 1.34) where as the prevalence of antibodies to the arbovirus was significantly higher in FR animals ( $P = 0.05$ , RR 0.58). There was no evidence of exposure to the remaining 33 viruses listed in Table 1.

All but one of FR orangutans ( $n = 34$ ) tested were positive for antibodies against one or more serotypes of *L. interrogans* (Table 6). Antibody titers of 1:1,600 were detected in two individuals. Antigen 85 levels indicated that SC orangutans had increased exposure to *Mycobacterium* spp. activity than did their FR counterparts (Kilbourn et al., 2001). *Burkholderia pseudomallei*, *Klebsiella* sp., *Mycobacterium avium*, and *Escherichia coli* were isolated from semi-captive orangutans during this study period.

The rate of *Plasmodium* spp. infection in SC orangutans (77%) was significantly higher ( $P < 0.01$ ) than in FR orangutans (24%) (Wolfe et al., 2001). Positive status was independent of sex ( $P > 0.05$ ) but not independent of age group ( $P < 0.01$ ) (Wolfe et al., 2001). No microfilaria were found in blood samples.

TABLE 4. Serum soluble elements in free-ranging orangutans from Sabah, Malaysia ( $n=33$ ).

Minerals (ppm dry weight)	Mean	SD	Range
B	1.38	0.16	1.11–1.67
Ba	0.07	0.01	0.05–0.13
Ca	99.2	8.18	88–125
Co	0.14	0.01	0.11–0.16
Cr	0.27	0.03	0.22–0.33
Cu	2.09	0.44	1.48–3.37
Fe	2.08	1	0.55–4.76
K	190	37.7	142–250
Mg	24	6.9	10.1–44.5
Mn	0.09	0.10	0.06–0.62
Mo	0.28	0.03	0.22–0.33
P	126.8	27.56	73.2–195
Zn	1.23	0.37	0.5–2.0

Fecal samples ( $n=28$ ) from the FR group were 14, 71, and 28% positive for *Balantidium coli*, *Strongyloides* sp., and *Trichuris* sp., respectively. All animals sampled had subjectively low levels of nematode infections based on less than one parasite ova per low power field.

Although most SC animals were treated with doramectin (Dectomax®, Pfizer Inc., New York, New York) and/or ivermectin (Ivomec® 1% MSD—Ag Vet, Merck and Co., Inc., Merial Limited, Iselin, New Jersey) monthly during the same period the FR were evaluated, 15% of samples were positive for *Strongyloides* sp., <5% positive for *Enterobius* sp., and 6.5% for *Trichuris* sp. The predominant organism, which appeared associated with morbidity in juvenile SC orangutans (pers. obs.), was *Balantidium coli* and prevalence was as high as 42%. Animals frequently remained positive despite treatment with 50 mg/kg metronidazole (Searle & Co., Skokie, Illinois, USA) administered orally.

#### DISCUSSION

With few exceptions, hematology, serum chemistry, and vitamin analyses were within ranges for captive orangutans (International Species Information System, 1997). Increased serum creatine kinase, lactate dehydrogenase, and aspartate aminotrans-

TABLE 5. Plasma vitamin levels ( $\mu\text{g/ml}$ ) in free-ranging orangutan in Sabah, Malaysia ( $n=38$ ).

Vitamin	Mean	SD	Range
Alpha-tocopherol	2.1	1	0.7–4.88
Gamma-tocopherol	0.27	0.12	0.1–0.55
Retinol	0.41	0.18	0.16–0.87

ferase (AST) in FR animals, especially individuals under 10 yr of age, may have been due to muscle trauma and exertion associated with capture and sample collection. Elevations in liver enzymes, alanine aminotransferase and AST, occurred predominantly in animals estimated to be <10 yr old. Elevation of these enzymes are generally associated with increased hepatocellular permeability (sublethal injury or necrosis; Duncan et al., 1994), but are not likely to be clinically significant. Use of PCBs and chlorinated pesticides is common in and around the large plantations where orangutans were captured; however, these compounds were not found in blood samples analyzed.

The serologic survey did not detect evidence of exposure to most of the non-arboviral agents tested in FR and SC orang-

TABLE 6. Results of serology for *Leptospira interrogans* in free-ranging orangutan in Sabah, Malaysia.

Serovar	Number positive/ number tested (%)
australis	4/34 (12%)
autumnalis	25/34 (73%)
ballum	9/34 (27%)
bataviae	0/34 (0%)
bratislava	16/34 (47%)
canicola	0/34 (0%)
grippotyphosa	30/34 (88%)
hardjo	2/34 (6%)
icterohaemorrhagiae/cop	4/34 (12%)
javanica	0/34 (0%)
pomona	0/34 (0%)
pyrogenes	2/34 (6%)
saxkoebing	1/34 (3%)
serjoe	2/34 (6%)
szwajizak	1/34 (3%)
tarassovi	0/34 (0%)
wolffi	3/34 (9%)



utans but there was exposure to six of eight arboviruses in both groups (Tables 1 and 2). In comparison, captive orangutans surveyed in the US have been exposed to a greater number of non-arboviruses (Kalter et al., 1997). Evidence of exposure to some non-arboviral pathogens was higher in SC than in FR orangutans. The prevalence of exposure to multiple potential pathogens in SC population is of concern.

The significant difference in evidence of exposure to adenovirus ( $P < 0.0005$ , RR 7.36, odds ratio [OR] 9) and rotavirus (SA 11) ( $P < 0.007$ , RR 2.04, OR 3.42) in SC compared to FR orangutans could be attributed to increased population densities of animals in captivity compared to FR animals and associated increased aerosol or fecal-oral transmission. These factors could also affect exposure to other respiratory and enteric viruses, which was lower or undetectable in FR orangutans compared to SC animals. Exposure to these viruses was not as common in SC animals compared to orangutans in the US. Evidence of exposure to these viruses in the US may be higher due to increased human contact, samples selected for analysis, older population of animals tested, or vaccination (Kalter et al., 1997).

Morbidity and mortality due to hand, foot and mouth disease in children in Malaysia in 1997 may have been caused by a coxsackie-B virus (Lum et al., 1998) similar to that tested for in the orangutans. The positive results in a few orangutans is probably due to cross-reactions in testing due to low specificity (Heberling, pers. comm.). Evidence of exposure of orangutans to mumps virus increased significantly in 1998. Mumps also increased in humans in Malaysia and surrounding countries (Goh, 1999) possibly associated with a vaccination program.

Lack of antibodies to hepatitis B virus (HBV), hepatitis A virus, simian retrovirus, herpes simplex virus, and simian immunodeficiency virus is notable when compared to the high prevalence reported in other populations of captive orangutans

undergoing rehabilitation (Warren et al., 1999). Forty-three percent of orangutans in East Kalimantan, Indonesia had antibodies to HBV. This virus was different than human HBV genotypes (Warren et al., 1999).

Exposure to dengue and Japanese encephalitis viruses appears high. There has been an increase in human cases of these diseases reported in Malaysia (Tesh et al., 1975; Anonymous, 1998a, b, 1999). If effects of these diseases in orangutans are similar to those in humans, exposure to these viruses could be a problem. The ubiquitous presence of antibodies to the Epstein Barr virus was similar to the high prevalence (80%) in captive orangutans in the US (Kalter et al., 1997). Continued work is needed to elucidate its impact on these primates.

There was a human outbreak of leptospirosis in Borneo (Anonymous, 2000). High antibody titers were detected in two orangutans. Single high titers may indicate active infection, but evaluation of significance requires testing serial samples.

Prevalence of *Plasmodium* spp. was significantly different between the two groups (Wolfe et al., 2001). The SC animals had significantly higher frequency of *Plasmodium* spp. infection (77%,  $P < 0.01$ ) than FR orangutans (24%). Positive status was independent of sex ( $P > 0.05$ ) but not independent of age group ( $P < 0.01$ ) (Wolfe et al., 2001). The SC population's high prevalence of *Plasmodium* spp. infections and low prevalence of positive arthropod-borne viral antibody titers were potentially influenced by animals' densities (SC  $> 100/\text{km}^2$ ; FR  $1 - < 20/\text{km}^2$ ) and presence of vectors. Host population density does not normally weigh heavily in estimates of vectorial capacity; there is only a linear increase in vectorial capacity associated with increases in host density (Spielman and James, 1990). The magnitude of change in host population density here, however, may have had a significant impact on vectorial capacity (Wolfe et al., 2001).

As discussed by Wolfe et al. (2001), high

prevalence of *Plasmodium pitheci* seen in the predominantly juvenile population of SC compared to FR animals, also could be explained by immature, naïve, and/or compromised immune systems of these young orangutans. Significant differences in age structure ( $P=0$ ) with a high proportion of juveniles among SC (87%, 112 of 129) versus adults in the FR population (38%, 32 of 84), may have influenced immunological status in the population.

Exposure of orangutans to humans occurs daily through food handling and presence of staff and tourists in close proximity to the animals and their environment. Human contact and other stressors, such as overcrowding, abnormal social structure or dietary imbalances, also exacerbate the potential susceptibility and transmission.

Treatment of disease in FR orangutans is impractical. In FR populations, as with SC ones, disease prevention is key to reducing adverse impacts on health. Reduction or prevention of disease in the SC could be accomplished by decreasing density, reducing interspecies contact, and modifying husbandry techniques and facilities management to reduce direct disease transmission, vectors, and fomites. Results from FR orangutans in this survey provide information that could help monitor impacts of environmental change (natural and anthropogenic) and translocation procedures.

Orangutans should not be translocated from high disease-risk or infected populations to areas where the receiving population is naïve or has low risk of disease exposure, depending on the pathogen. Animals infected with a disease should not be moved to a release site where the disease does not exist. Some infectious agents ubiquitous in FR and SC populations and not associated with clinical disease, such as Epstein-Barr virus, may not warrant special concern with regards to the translocation of animals from one setting to another. Elimination of nonessential interspecies contact and reduction of vector-borne disease transmission is recommended in SC animals.

Preventative medicine and health evaluations should be incorporated into all future orangutan translocation and rehabilitation procedures. Information resulting from monitoring should be used to modify wildlife management techniques.

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