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EXPLORING THE ECOLOGIC BASIS FOR EXTREME SUSCEPTIBILITY OF PALLAS' CATS (*OTOCOLOBUS MANUL*) TO FATAL TOXOPLASMOSIS

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ABSTRACT: Recent efforts by North American zoos to establish a genetically viable captive population of Pallas' cats (*Otocolobus manul*) have been compromised by high newborn mortality (~60%), primarily because of toxoplasmosis. The basis for this extreme susceptibility to toxoplasmosis is unknown. In the present study, the general health status of wild Pallas' cats in Mongolia was evaluated, including assessment of basal hematologic parameters and fecal corticoid metabolite concentrations. The prevalence of exposure to *Toxoplasma gondii* in Mongolian Pallas' cats, local domestic cats, and prey species also was determined based on serology and/or polymerase chain reaction analysis. Biologic samples (blood, feces, and/or brain tissue) were obtained from 15 wild Pallas' cats, 15 domestic cats, and 45 prey animals (rodents and pikas) captured in Mongolia during the summers of 2000 and 2001. Comparative data were obtained from nine captive Pallas' cats maintained in North American zoos. Based on physical examinations, complete blood counts, and blood chemistry analyses, only minor differences were observed in the general health status of wild and captive Pallas' cats. Fecal cortisol metabolite concentrations did not differ ($P>0.05$) between populations, indicating that Pallas' cats in captivity and in the wild have similar basal adrenocortical activity. A pronounced difference ($P<0.01$) in seroprevalence to *T. gondii* was observed between populations. Whereas all captive Pallas' cats exhibited elevated immunoglobulin titers (IgG>2,048) to *T. gondii,* only two of 15 (13%) wild Pallas' cats were seropositive, with both cats having lower IgG titers (<1,024). Furthermore, no evidence of exposure to this parasite was found in any of the Mongolian domestic cats or prey species. These findings suggest that wild Pallas' cats have minimal opportunity for exposure to *T. gondii* in their natural habitat and, typically, do not become infected with this parasite until being brought into captivity. Accordingly, maintenance of a viable captive population may require implementing effective strategies to prevent exposure of immunologically naive Pallas' cats to *T. gondii* and to reduce parasite transmission between seropositive females and their highly susceptible offspring.

Key words: Blood chemistry, captive, free-ranging, hematology, Mongolia, Pallas' cats, serology, *Toxoplasma gondii*.

INTRODUCTION

The Pallas' cat (*Otocolobus manul*), a small-sized felid species that is endemic to Central Asia, has numerous biologic adaptations reflective of the harsh winters and distinct seasonal changes encountered in its natural environment. Taxonomically, the Pallas' cat is classified as the sole representative of its genus (*Otocolobus*) and is noted for its long fur, stocky build, and flattened face (Nowell and Jackson, 1996). Other adaptations include a pronounced reproductive seasonality, with females typically showing estrous cyclicity only during the winter months and producing a single litter each year, with births occurring in late spring to early summer (Swanson et al., 1996; Brown et al., 2002). Few studies have investigated the ecology of wild Pallas' cats, and virtually no information is available regarding epidemiologic, hematologic, or other physiologic characteristics of the wild population.

Pallas' cats are considered to be threat-

ened with extinction in their natural habitat (Convention on International Trade in Threatened Species, 2001), primarily because of habitat loss, vermin control programs, and hunting for the fur trade (Nowell and Jackson, 1996). To establish a genetically representative founder population in captivity, 24 wild-born Pallas' cats were imported from Russia and Mongolia for North American zoos from 1995 to 2000, and the American Zoo and Aquarium Association initiated a Species Survival Plan in 2001. Captive breeding of these Pallas' cat founders has been very productive, with the birth of 65 kittens in 17 litters between 1996 and 2001. Newborn kittens, however, have experienced extraordinarily high (~60%) mortality within 4 mo of birth. In the majority of deceased kittens evaluated at necropsy, infection with *Toxoplasma gondii* was identified as the cause of death (Swanson, 1999; Kenny et al., 2002).

Almost all adult Pallas' cats (>80%) in North American zoos are chronically seropositive for anti–*T. gondii* immunoglobulins (Swanson, 1999) and, like most adult felids, only rarely have clinical signs of infection (Reimann et al., 1974; Dubey et al., 1988; Kenny et al., 2002). During pregnancy, however, maternal immune responses in seropositive females apparently are not protective in Pallas' cats, unlike the case in pregnant domestic cats and other cat species with previous exposure to *T. gondii* (Swanson, 1999; Kenny et al., 2002). High neonatal mortality in Pallas' cats is jeopardizing the maintenance of a viable captive population, but the basis for the extreme susceptibility of this species to fatal toxoplasmosis is unknown. Comparative information from wild Pallas' cats would be invaluable for determining whether toxoplasmosis is endemic in wild populations or is associated primarily with captivity.

In the present study, specific objectives were 1) to evaluate the general health status of wild Pallas' cats in Mongolia, including normative blood values, viral antibodies and antigenemia, and fecal parasite loads; 2) to determine prevalence of exposure to *T. gondii* in Mongolian Pallas' cats, domestic cats, and prey species; 3) to assess fecal cortisol metabolite levels as an indirect indicator of basal stress; and 4) to compare findings to those from concurrent studies of wild-born Pallas' cats maintained in North American zoos.

MATERIALS AND METHODS

In Mongolia, biologic samples were collected from 15 wild Pallas' cats (five adult females, 2.3–3.2 kg; four adult males, 1.4–3.1 kg; four juvenile females, 0.8–1 kg; two juvenile males, 0.9–1 kg), 15 adult domestic cats, and 45 prey animals (25 small rodents, *Lasio podomys brandti*; seven gerbils, *Meriones unguiculatus*; seven gray rodents, *Alticola argentatus*; six pikas, *Ochotona mongolica*). For comparison, biologic samples also were collected from nine adult Pallas' cats (four wild-born females, four wild-born males, one captive-born female) housed in US zoos. Blood samples were obtained opportunistically from captive animals during routine annual or bi-annual health examinations of each zoo's resident Pallas' cats.

Biologic samples were obtained from wild adult and juvenile Pallas' cats captured in the central province of Mongolia (47°23′50.2″N, 106°02′02.7″E) during the summers of 2000 and 2001. For capture, active Pallas' cat den sites were identified by visual observation and excavated by shoveling. Pallas' cats were restrained manually using leather gloves, placed into wire holding cages, and anesthetized (ketamine hydrochloride, 15 mg/kg body mass intramuscularly; Fort Dodge, Fort Dodge, Iowa, USA) by hand syringe. Blood (8–12 ml) was collected via jugular venipuncture, divided between ethylenediaminetetraacetic acid (EDTA) and clot tubes, and centrifuged at 1,100 × G for 10 min (Mobilespin, Vulcon Technologies, Grandview, Missouri, USA) within 24 hr of collection. Plasma, red and white blood cells, whole blood, and serum samples were aliquoted into labeled cryovials, frozen in liquid nitrogen vapor, and stored in a liquid nitrogen tank (−196 C). A separate whole-blood sample (1 ml in EDTA) was chilled (4 C) for CBC analysis within 24 hr of collection. Fecal samples were obtained following voluntary defecation of restrained cats or from the rectum via a lubricated rectal loop. Half the stool sample was preserved in polyvinyl alcohol and cupric sulfate (Para-Pak Modified PVA, Meridian Diagnostics, Cincinnati, Ohio, USA), and the remainder was stored frozen in a labeled cryovial

(−20 C). External parasites were collected, fixed in alcohol, and stored at room temperature in a labeled tube. When fully recovered from anesthesia (after ~2 hr), cats were released close to their point of capture. For comparison, biologic samples also were obtained from captive Pallas' cats maintained in three North American zoos (Birmingham Zoo, Birmingham, Alabama; Cincinnati Zoo & Botanical Garden, Cincinnati, Ohio; Denver Zoological Gardens, Denver, Colorado). Blood and fecal samples were collected during 1999–2000 and subjected to similar laboratory analyses (described below).

Blood samples also were collected from domestic cats (*Felis silvestris catus*) captured at the study sites in Mongolia, nearby villages, and in Ulaanbaatar during the summer of 2001. Cats were anesthetized (ketamine hydrochloride, 15 mg/kg body mass intramuscularly) and blood (2–4 ml) was collected via venipuncture, placed into EDTA tubes, and centrifuged at 1,100 × G for 10 min. Plasma and whole blood from each cat were aliquoted into labeled cryovials, frozen, and stored in a liquid nitrogen tank (−196 C). Various prey species (small rodents, gerbils, and pikas) were captured manually by local Mongolian herdsmen. For anesthesia, each animal was placed into a nylon bag containing a cotton ball soaked with halothane (Halocarbon Laboratories, River Edge, New Jersey, USA). Blood samples (2 ml) were obtained via cardiac puncture, placed into EDTA tubes, and centrifuged (1,100 × G for 10 min). Whole blood and plasma were aliquoted into labeled cryovials and stored in liquid nitrogen (−196 C). Immediately following blood sample collection, anesthetized prey animals were killed by decapitation. Whole brains were isolated, sectioned, and stored in buffered formalin for polymerase chain reaction (PCR) analysis.

Sample analysis included assessments of blood cell and biochemistry parameters, serum antibodies against and antigens of select viral agents, serum antibodies against *T. gondii,* PCR assay for *T. gondii* DNA, fecal flotation, and fecal cortisol metabolite concentrations. Complete blood counts (CBCs) on fresh (<24 hr) chilled samples from wild cats were performed at the MCS Gyals Medical Center in Ulaanbaatar, Mongolia, using an automated cell counter (PCE-140 Particle Counter, Erma, Inc., Tokyo, Japan) and manual differential staining. For CBCs of captive cats, fresh (<12 hr) whole-blood samples were assessed either at the local veterinary diagnostic laboratory using an automated cell counter (Avid Cell-Dyn 3500, Abbott Laboratories, Abbott Park, Illinois, USA) with manual differentials or by zoo hospital staff using blood dilution reserviors (Unopette, Becton Dickinson and Company, Franklin Lakes, New Jersey, USA) and hemocytometers for manual counts. Biochemistry analysis (Hitachi 717 Clinical Chemistry Analyzer, Roche Diagnostics, Indianapolis, Indiana, USA) and enzyme-linked immunoassays (ELISA) for feline immunodeficiency virus (FIV; Petchek FIV ELISA, Idexx Laboratories, Westbrook, Maine, USA) and coronavirus (Virachek CV, Synbiotics Corp., San Diego, California, USA) antibodies and feline leukemia virus (FeLV) antigens (FeLV ELISA, Hansen Veterinary Immunology, Dickson, California, USA) were conducted at the Cincinnati Veterinary Lab using frozen-thawed serum samples. Zinc sulfate fecal flotation for *T. gondii* oocysts was conducted at the Ohio State University College of Veterinary Medicine (Bowman, 1999).

Enzyme immunoassay (EIA) and latex agglutination testing of plasma samples for *T. gondii* immunoglobulins and PCR testing of whole blood (all animals) and brain tissue (prey species) for *T. gondii* DNA were conducted at Colorado State University (Homan et al., 2000; Lappin et al., 1991). Briefly, tissue (~25 mg) was sectioned from formalin-fixed brain of each prey animal using separate instruments to avoid potential DNA contamination and then placed into PCR-ready microcentrifuge tubes (Qiagen, Inc., Valencia, California, USA) for DNA extraction, which was performed using a commercially available kit (DNeasy Tissue Kit, Qiagen). For an internal negative control, water was digested every 10 samples using the tissue extraction method. The positive control was fresh brain tissue from a *T. gondii*–infected mouse. In other experiments (data not shown), the sensitivity of this PCR assay was shown to be equivalent between fresh and formalin-fixed murine brain tissue containing *T. gondii* bradyzoites. For whole-blood samples, DNA was extracted with a commercially available kit (QIAamp DNA Blood Mini Kit, Qiagen). *Toxoplasmosis gondii* DNA from digested tachyzoites was used as the positive control. *Toxoplasmosis gondii* PCR was performed using primers TOX4 and TOX5, as described previously (Homan et al., 2000), with the following modifications: The 50-μl reaction mix consisted of 10 mM Tris-HCl (pH 8.3), 50 mM KCl (Perkin-Elmer, Inc., Wellesley, Massachusetts, USA), 2.5 mM $MgCl_2$, 0.5 μM of each primer, 200 μM of each dNTP (Promega Corp., Madison, Wisconsin, USA), and 2 U of gold Taq polymerase (Perkin-Elmer). Amplification was performed on a Perkin-Elmer 480 thermal cycler using a time-release PCR assay protocol as follows: 95 C for 5 min, then 40 cycles of 95 C

for 1 min, 55 C for 1 min, and 72 C for 30 sec, with an extra 1 sec added onto each cycle, followed by a final extension of 72 C for 5 min and a 4 C hold. The amplified products (529 bp) were observed by electrophoresis in a 1.5% agarose gel containing ethidium bromide.

For cat plasma samples, *T. gondii* IgM and IgG were detected as follows: An optimal dilution of *T. gondii* tachyzoite antigens in 0.06 M carbonate buffer (pH 9.6) was pipetted (100 μl) into wells of a micro-ELISA plate (Immulon I, Kirkegaard and Perry Laboratories, Gaithersburg, Maryland, USA) and incubated at 4 C overnight. Following three plate washes with 0.01 M phosphate-buffered saline solution (PBSS) plus 0.05% Tween 20 (PBSS-TW), each plasma sample was diluted 1:64 in PBSS-TW and pipetted into triplicate wells (100 μl/well), and the plate was incubated for 30 min at 37 C. After three washes, 100 μl of a 1:3,000 dilution of anti-feline IgM (peroxidase labeled, heavy-chain specific, Kirkegaard and Perry) or 100 μl of a 1:2,000 dilution of anti-feline IgG (peroxidase labeled, heavy-chain specific, Kirkegaard and Perry) were pipetted into appropriate wells and the plate incubated for 30 min at 37 C. After three washes, 100 μl of a substrate (SureBlue TMB microwell peroxidase substrate, Kirkegaard and Perry) were pipetted into appropriate wells and the plate incubated for 10 min at 20 C. To stop the color reaction, 100 μl of 0.18 M H_2SO_4 was added to each well, and absorbances were then calculated by an automated micro-ELISA plate reader using a 450-nm filter. Positive-control, negative-control, enzyme-control, and substrate-control wells were included on each plate. The mean absorbance of each serum sample was compared to a standard curve developed from negative- and positive-control sera, and a titer was assigned. Positive cutoff absorbances were defined as greater than the mean plus two SDs of results from 20 specific pathogen-free domestic cats. Titers of 64 or greater were considered to be positive. Positive ELISA results were confirmed via latex agglutination using a commercially available latex agglutination kit following instructions supplied by the manufacturer (TOXOTEST-MT*Eiken*, Tanabe USA, Inc., San Diego, California, USA). Similarly, *T. gondii* immunoglobulins were detected in plasma from the prey species using latex agglutination (TOXOTEST-MT*Eiken*). Because this assay is not immunoglobulin class specific, all classes of immunoglobulins (i.e., IgG, IgM, etc.) should be detected. Titers of 64 or greater were considered to be positive.

Fecal extractions and corticosterone radioimmunoassays (RIAs) were performed at the National Zoo's Conservation and Research Center as described by Brown et al. (1994) with slight modification. Briefly, fecal corticoid metabolite immunoreactivity was measured on wet fecal samples using a double-antibody ^{125}I RIA kit for corticosterone (ICN Biomedicals, Inc., Costa Mesa, California, USA) validated previously for domestic cats. Frozen fecal samples were thawed, weighed (0.18–0.20 g/sample), and then boiled in 90% ethanol/distilled water (5 ml/sample) for 20 min. Following centrifugation at 2500 × G for 10 min, recovered supernatants were dried down in air, resuspended in methanol (1 ml), and diluted (1:5 to 1:40) in phosphate buffer (0.01 M $NaPO_4$, 0.14 M NaCl, 0.5% BSA, and 0.01% NaN_3; pH 7.4) for RIA. Samples were assayed in duplicate and the results expressed as ng/g wet feces.

For data analysis, the mean±SD was calculated for biochemistry and hematologic parameters and fecal corticoid levels, and differences were assessed between wild and captive populations using an unpaired *t*-test (Statview, SAS Institute, Cary, North Carolina, USA). The proportion of wild and captive Pallas' cats that were seropositive to *T. gondii* were compared using a Fisher exact test (Statview). Probability values of less than 0.05 were considered to be significant.

RESULTS

No significant differences ($P>0.05$) were observed in any hematologic values or in most biochemistry parameters among wild and captive Pallas' cat populations (Table 1) with the following exceptions: Wild Pallas' cats had higher ($P<0.05$; mean ± SD) serum aspartate aminotransferase (AST, 119.4±59.5 IU/l) and total bilirubin (0.70±0.39 mg/dl) and lower ($P<0.05$) glucose (47.0±32.4 mg/dl) levels compared to those found in captive Pallas' cats (35.8±36.5 IU/l, 0.19±0.16 mg/dl, and 121.1±28.7 mg/dl, respectively). Viral screening via domestic cat ELISA kits revealed no evidence of FIV, FeLV, or coronavirus in the wild or captive populations.

Of the 15 wild Pallas' cats, 13 (87%) were negative for anti–*T. gondii* immunoglobulins via EIA (Table 2). The two seropositive cats had IgG titers of 512 and 1,024, respectively. These two positive results were confirmed via latex agglutination. In contrast, all (9/9) of the compar-

TABLE 1. Serum biochemistry and hematologic values for wild (n=15) versus captive (n=9) Pallas' cats.

Parameter[a]	Wild Pallas' cats (n=15)		Captive Pallas' cats (n=9)	
	Mean ± SD	Range	Mean ± SD	Range
AST (IU/l)	119±59.5*	62–248	35.8±36.5*	14–117
ALT (IU/l)	73.4±21.3	49–119	63.6±36.0	22–112
Total bilirubin (mg/dl)	0.70±0.39*	0.12–1.28	0.19±0.16*	0.1–0.54
BUN (mg/dl)	44.2±23.5	22.7–98.3	44.2±14.7	37–73.9
Creatinine (mg/dl)	0.81±0.17	0.5–1.1	1.2±0.405	0.73–2
BUN:creatinine ratio	45.6±17.2	23.2–80.5	36.8±5.65	26.8–43.5
Cholesterol (mg/dl)	125±35.4	85–197	125±26.9	94–159
Alkaline phosphatese (IU/l)	54.4±60.1	1–133	5.7±1.47	3.3–7
Glucose (mg/dl)	47±32.4*	13–106	121.1±28.7*	61.3–149
Phosphorus (mg/dl)	7.0±2.61	4.4–11.2	5.2±0.743	4.1–6.3
Calcium (mg/dl)	9.3±1.64	5.4–11	8.3±0.781	7.4–9.4
Calcium:PO_4 ratio	1.4±0.40	0.8–2	1.6±0.342	1.2–2
Total protein (g/dl)	6.7±0.92	4.3–7.7	6.3±0.116	6.2–6.5
Albumin (g/dl)	3.9±0.60	2.5–4.7	3.4±0.132	3.2–3.6
Globulin (g/dl)	2.9±0.54	1.8–3.6	2.9±0.212	2.7–3.2
LDH (IU/L)	1,090±800	170.6–2,000	84.9±88.7	27–234.3
Sodium (mEq/l)	160±2.40	156–163	156.6±1.48	155–159.5
Potassium (mEq/l)	4.7±18.8	3.5–5.4	4.2±0.251	4–4.7
Chloride (mEq/l)	119±7.90	109–131	119.8±5.29	116–130
WBCs ($\times 10^3/\mu l$)	11.1±3.68	6.3–16.7	9.4±2.64	5.8–13.3
RBCs ($\times 10^6/\mu l$)	7.1±1.70	3.5–9.1	6.8±0.528	6.2–7.1
Hemoglobin (g/dl)	11.4±0.849	10.3–13.6	11.3±1.06	9.3–13
Hemotocrit (%)	36.8±3.68	29.7–43.8	29.3±2.38	25.1–33
MCV (fl)	57.9±24.9	39.3–111.7	43.3±3.70	40.5–50.9
MCH (pg)	17.8±6.79	12.4–32.3	16.8±1.85	15.1–20.3
MCHC (g/dl)	31.4±5.09	25.8–45.7	38.7±1.06	37.3–39.9
PLT ($\times 10^5/\mu l$)	144.3±122	6–406	N/A[b]	N/A
Segs ($\times 10^3/\mu l$	6,636.5±2,400	3,668–13,035	5,607±1,639.4	3,859–7,980
Bands ($\times 10^3/\mu l$)	73.7±82.07	0–250	0	0
Lymphs ($\times 10^3/\mu l$)	2,419.1±1,007	378–4,500	2,775±1,440	1,060–4,788
Monophils ($\times 10^3/\mu l$)	387.4±34	0–1,169	196±140	0–399
Eosinophils ($\times 10^3/\mu l$)	1,297±1,251	63–4,396	186±219	0–574
Basophils ($\times 10^3/\mu l$)	125.3±317	0–1,002	0	0

[a] ALT = alanine aminotransferase; AST = aspartate aminotransferase; BUN = blood urea nitrogen; LDH = lactate dehydrogenase; MCH = mean cell hemoglobin; MCHC = mean cell hemoglobin concentration; McV = mean cell volume; PLT = platelets; RBCs = red blood cells; Segs = segmented neutrophils; WBCs = white blood cells.

[b] N/A = not available.

* Mean values differ significantly (P<0.05).

ative captive population were seropositive for *T. gondii,* with IgG titers ranging from 2,048 to 8,192. Two of the captive cats also had anti–*T. gondii* IgM titers, whereas *T. gondii* IgM was undetectable in any of the wild cats (Table 2). Assessment of immunoglobulin titers in sera from Mongolian domestic cats and prey species indicated a complete absence of exposure to *T. gondii.* Furthermore, PCR analysis of whole-blood samples from all wild Pallas' cats (Table 2), domestic cats, and prey items as well as of brain tissue from prey species found no evidence of *T. gondii* DNA.

Comparison of fecal corticoid metabolite concentrations revealed no significant difference (P>0.05) between wild (46.89±8.53 ng/g wet feces) and captive (56.82±7.93 ng/g wet feces) Pallas' cats (Table 3). *Toxoplasmosis gondii* oocysts were not found in fecal samples from the wild Pallas' cats. *Isospora* oocysts and *Tox-*

TABLE 2. Immunoglobulin titers and polymerase chain reaction (PCR) results for *Toxoplasma gondii* in wild (n=15) versus captive (n=9) Pallas' cats[a].

Cat no.	*Toxoplasma* IgM titer	*Toxoplasma* IgG titer	Toxoplasma PCR	Toxoplasma LA	Cat no.	*Toxoplasma* IgM titer	*Toxoplasma* IgG titer	Toxoplasma PCR
1	N	1024	N	P	1	N	2048	N
2	N	N	N		2	N	4096	N
3	N	N	N		3	N	4096	N
4	N	N	N		4	N	4096	N
5	N	N	N		5	64	2048	N
6	N	N	N		6	N	4096	N
7	N	N	N		7	N	8192	N
8	N	N	N		8	64	4096	N
9	N	512	N	P	9	N	8192	N
10	N	N	N					
11	N	N	N					
12	N	N	N					
13	N	N	N					
14	N	N	N					
15	N	N	N					
Total	0/15	2/15*	0/15			2/9	9/9*	0/9

[a] LA = Latex agglutination; N = negative; P = positive.
* Values differ significantly (P<0.01).

ocara eggs were found in four of the fecal samples collected from wild cats, and ticks (*Rhipicephalus* spp.) and fleas were found on six wild cats.

DISCUSSION

To our knowledge, the present study is the first detailed investigation of biomedical parameters in wild Pallas' cats, and its findings may have broad implications for the management of both captive and wild Pallas' cat populations. The present results suggest that the general health status of wild and captive Pallas' cats is comparable, based on CBCs and blood biochemistry analyses. With the exception of serum AST, total bilirubin, and glucose, no significant differences were noted between populations in hematologic or biochemistry parameters. The few biochemistry differences most likely were artifacts of sample handling and/or were influenced by

TABLE 3. Fecal corticosterone levels (ng/g wet feces) as an indicator of adrenocortical activity in wild (n=11) versus captive (n=9) Pallas' cats.

Wild		Captive	
Cat no.	Corticosterone (ng/g wet feces)	Cat no.	Corticosterone (ng/g wet feces)
Mong3	30.62	Cap1	93.02
Mong4	76.24	Cap2	64.96
Mong5	56.20	Cap3	27.51
Mong6	53.09	Cap4	42.73
Mong7	13.09	Cap5	46.18
Mong9	42.01	Cap6	50.34
Mong10	36.08	Cap7	71.57
Mong11	115.44	Cap8	28.24
Mong12	33.72	Cap9	86.80
Mong13	29.15		
Mong14	30.16		
Mean ± SD	46.89±8.53		56.82±7.93

the relative ages of individuals in each population. Serum from at least four of the wild Pallas' cats had evidence of hemolysis, which can elevate aspartate aminotransferase and total bilirubin values falsely and decrease blood glucose levels (Narayanan, 2000). In addition, all the captive animals sampled in the present study were adults, whereas six of the 15 wild Pallas' cats were juveniles (age, <9 mo), possibly contributing to the differences noted in serum glucose levels (Narayanan, 2000).

Antibodies against FIV and coronavirus and the presence of FeLV antigen were assessed in serum using commercially available domestic cat ELISAs. All the wild Pallas' cats were found to be seronegative by use of these assays. When the sera were examined by Western blot immunoassay for FIV antibodies, however, four wild Pallas' cats were found to be positive (Troyer et al., 2005), which to our knowledge was the first report of FIV from free-ranging Asian cats. In captivity, Pallas' cats are potential hosts for FIV, with both confirmed (Barr et al., 1997) and suspected (Ketz-Riley et al., 2003) cases reported in zoos. One FIV variant isolated from a captive Pallas' cat, which had been born wild in Kazakhstan, has been well characterized by both DNA sequencing and cytopathic effects during cell culture (Barr et al., 1995, 1997). Research is ongoing to obtain and characterize the FIV DNA sequence from the wild Mongolian Pallas' cats for comparison to the captive Pallas' cat FIV DNA sequences reported by Barr et al. (1995, 1997).

As one indicator of basal adrenal activity, Pallas' cat fecal samples were analyzed for corticoid metabolites, a technique that has been validated as a noninvasive method for assessing responses to stressful stimuli in many species, including felids (Graham and Brown, 1996; Wasser et al., 2000). In the present study, fecal corticoid metabolite concentrations did not differ between wild and captive populations, indicating that Pallas' cats in captivity and in the wild exhibit similar levels of adrenocortical activity. In the only other published study comparing wild and captive felid populations, fecal corticoid levels in captive cheetahs were shown to be elevated relative to those in their wild counterparts (Terio et al., 1999). Those authors concluded that chronic stress might be responsible, in part, for the high incidence of *Helicobacter* sp.–related gastritis observed in the captive population. In contrast, the present results do not support the hypothesis that chronic stress is causing the extreme susceptibility of Pallas' cats to toxoplasmosis. Numerous variables, however, such as content of diet, availability of food and water, gender, age, season, and sample collection as well as storage methods, can affect cortisol values measured in fecal samples (Millspaugh and Washburn, 2004). Further investigation regarding the impact of these variables on cortisol levels and the relationship of stress to disease susceptibility in Pallas' cats may be warranted.

One pronounced difference observed between wild and captive populations was the seroprevalence to *T. gondii.* All captive Pallas' cats evaluated in the present study had *T. gondii* IgG titers of greater than 2,048, and two were also positive for *T. gondii* IgM, suggesting recent exposure to the parasite (Lappin, 1996). In an earlier survey of North American zoos (Swanson, 1999), 79% (11/14) of wild-born adult Pallas' cats had *T. gondii*–specific antibody titers when tested within 60 days of importation from Russian zoos. In contrast, in the present study, only two of 15 (13%) wild Pallas' cats were found to be seropositive for *T. gondii*–specific antibody. An additional four wild-born Pallas' cats imported directly from Mongolia in 2000 also were seronegative on arrival (John Aynes, pers. comm.). Although the presence of two seropositive wild Pallas' cats confirms that *T. gondii* is indigenous to Mongolia, seroprevalance data suggest that most wild Pallas' cats have minimal opportunity for exposure to this parasite in their natural habitat. Accordingly, we suspect that most

wild-born Pallas' cats become infected with *T. gondii* only after being brought into captivity.

Results from our concurrent evaluations of Mongolian domestic cats and prey species support this conclusion. No evidence of *T. gondii* antibodies was observed in any of the domestic cats, pikas, rodents, or gerbils sampled in Mongolia. Furthermore, PCR analysis of brain tissue obtained from 45 prey animals failed to identify *T. gondii* in any animal tested. In contrast, studies in other geographic regions have reported *T. gondii* seroprevalence ranging from 9% to 85% in nondomestic cat species, from 33.7% to 80% in domestic cats, and from 2.1% to 24% in prey species (Roelke et al., 1993; Dubey et al., 1995; Hejlicek and Nezval, 1997; Hill et al., 1998; Silva et al., 2001; Defeo et al., 2002). Further research is warranted to substantiate our findings.

Broader biomedical surveys of wild Pallas' cats, domestic cats, prey species, and alternate intermediate hosts, including larger mammals and livestock, are needed in Mongolia and Russia to expand the seroprevalence data and to search for other potential *T. gondii* reservoirs. In addition, comparative assessments should be conducted in other wild felids (snow leopard, *Panthera uncia*; lynx, *Lynx lynx*; wild cat, *Felis silvestris*) that are endemic to this region. No evidence suggests a similar susceptibility of these species to toxoplasmosis in zoos, but few wild-born individuals have entered captivity in recent years.

The available evidence suggests that the extreme susceptibility of Pallas' cats to toxoplasmosis is a consequence of evolving in a biologically unique environment. In their culture, Mongolians typically do not maintain domestic cats as companion animals, limiting the number of feral cats available to serve as definitive hosts for *T. gondii.* Persistence of *T. gondii* is dependent on the presence of felids in the environment (Wallace, 1969), and low domestic cat densities are associated with low *T. gondii* prevalence (Dubey et al., 1997). Furthermore, the severity of the winters and high altitude likely reduce the viability of any *T. gondii* oocysts that may be shed into the environment by domestic cats and other wild felids in Mongolia. The *T. gondii* oocysts have poor survivorship at both extreme temperatures and high altitudes (Dubey and Beattie, 1988). The combination of limited numbers of definitive hosts and low oocyst survival during the winter months could impair the ability of *T. gondii* to complete its natural life cycle and persist in the wild. Accordingly, Pallas' cats may not have coevolved with this specific parasite, creating a degree of susceptibility to toxoplasmosis that is most similar to that observed in several island species, such as macropods and New World primates (Dubey, 1986; Dubey et al., 1991; Canfield et al., 1990), or that seen more recently in other immunologically naïve species, such as southern sea otters (Miller et al., 2002). The degree of susceptibility of Pallas' cats to toxoplasmosis is unique among felid species but entirely consistent with expected outcomes when naïve species are first exposed to a novel pathogen.

These findings may have profound implications for the Pallas' cats in captivity and, possibly, in the wild. If zoos are to maintain Pallas' cats in captivity, it is imperative that research be directed at developing management strategies to prevent exposure of naive Pallas' cats to *T. gondii* without compromising reproduction. Recent studies have demonstrated that Pallas' cats maintained in indoor exhibits under simulated natural photoperiods and fed a processed, *T. gondii*–free cat food diet exhibit typical seasonal reproductive patterns (Swanson and Kennedy-Stoskopf, 2002). For Pallas' cats previously infected with *T. gondii,* pharmaceutical options need to be explored that will limit transmission of disease to offspring in utero or postpartum and provide effective therapy for clinically ill neonates (Swanson et al., 2001; Kenny et al., 2002). Finally, the emergence of toxoplasmosis among wild Pallas' cat populations remains a possibility if cultural and ecologic factors (e.g., domestic cat

density and ambient temperatures) are altered in range countries, which could have devastating consequences if neonatal mortality rates are similar to those seen in captive populations.

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