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Authors: Loeffler, I. Kati, Howard, JoGayle, Montali, Richard J., Hayek, Lee-Ann, Dubovi, Edward, et al.

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SEROSURVEY OF EX SITU GIANT PANDAS (*AILUROPODA MELANOLEUCA*) AND RED PANDAS (*AILURUS FULGENS*) IN CHINA WITH IMPLICATIONS FOR SPECIES CONSERVATION

I. Kati Loeffler, D.V.M., Ph.D., JoGayle Howard, D.V.M., Ph.D., Richard J. Montali, D.V.M., Dipl. A.C.V.P., Dipl. A.C.Z.M., Lee-Ann Hayek, Ph.D., Edward Dubovi, Ph.D., Zhihe Zhang, M.S., Qigui Yan, D.V.M., Wanzhu Guo, M.S., and David E. Wildt, Ph.D.

Abstract: Conservation strategies for the giant panda (*Ailuropoda melanoleuca*) include the development of a self-sustaining ex situ population. This study examined the potential significance of infectious pathogens in giant pandas ex situ. Serologic antibody titers against canine distemper virus (CDV), canine parvovirus (CPV), canine adenovirus (CAV), canine coronavirus (CCV), canine herpesvirus, canine parainfluenza virus (CPIV), *Toxoplasma gondii*, *Neospora caninum*, and *Leptospira interrogans* were measured in 44 samples taken from 19 giant pandas between 1998 and 2003 at the Chengdu Research Base of Giant Panda Breeding in Sichuan, China. Seroassays also included samples obtained in 2003 from eight red pandas (*Ailurus fulgens*) housed at the same institution. All individuals had been vaccinated with a Chinese canine vaccine that included modified live CDV, CPV, CAV, CCV, and CPIV. Positive antibody titers were found only against CDV, CPV, and *T. gondii*. Sera were negative for antibodies against the other six pathogens. Results indicate that the quality of the vaccine may not be reliable and that it should not be considered protective or safe in giant pandas and red pandas. Positive antibody titers against *T. gondii* were found in seven of the 19 giant pandas. The clinical, subclinical, or epidemiologic significance of infection with these pathogens via natural exposure or from modified live vaccines in giant pandas is unknown. Research in this area is imperative to sustaining a viable population of giant pandas and other endangered species.

Key words: China, giant panda, infectious disease, red panda, serology.

INTRODUCTION

The free-living population of giant pandas (*Ailuropoda melanoleuca*) is estimated to be 1,600 individuals who live in highly fragmented habitat.[20] A genetically viable, self-sustaining captive population may serve as a hedge against extinction of the species. Significant progress has been made in the last decade in terms of understanding the nutrition, behavior, genetics, and reproduction of giant pandas, toward the goal of building the ex situ population.[21] There is little information, however, about the prevalence or types of infectious diseases in captive or wild giant pandas. The paucity of knowledge in this area may compromise our ability to protect the species.[11,12,16]

The only serologic investigation of giant pandas in China took place more than 15 yr ago and involved eight giant pandas housed at the Conservation and Research Center for Giant Pandas at the Wolong Nature Reserve in Sichuan Province.[17] Serologic data from local domestic dogs and cats were also examined. The study indicated that giant pandas in the captive breeding program and giant pandas that had recently been removed from the wild had been exposed to canine distemper virus (CDV), canine parvovirus-2 (CPV), canine adenovirus (CAV), and canine coronavirus (CCV).

Since that study, the ex situ giant panda population has more than doubled. There are now approximately 250 individuals worldwide, of which 90% are in China. Sixty percent of the Chinese population is currently located in just three facilities in central Sichuan province: 1) the Wolong Nature Reserve breeding center; 2) the Chengdu Research Base of Giant Panda Breeding (hereafter referred to as Chengdu Panda Base); and 3) the Bi Feng Xia Zoo, which is affiliated with the Wolong center. Giant pandas are moved among captive facilities and from the wild without quarantine or disease monitoring precautions. Like most captive wildlife in China, the giant pandas in these facilities are well within the radius of exposure to infectious diseases of other wildlife and domestic species.

Captive giant pandas in China present numerous and frequent signs of illness that may have an in-

From the Smithsonian's National Zoological Park, Washington, D.C. 20008, USA (Loeffler, Howard, Montali, Wildt); Smithsonian's National Museum of Natural History, Washington, D.C. 20560, USA (Hayek); Animal Health Diagnostic Center, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853, USA (Dubovi); Chengdu Research Base of Giant Panda Breeding, Chengdu, Sichuan 610081, China (Zhang); and the Animal Biotechnology Center, Sichuan Agriculture University, Ya'an, Sichuan 625014, China (Yan, Guo). Correspondence should be directed to Dr. Loeffler (katiloeffler@gmail.com).

fectious etiology. Adults and young often experience gastrointestinal and respiratory disease.[11,12] Neonates and cubs are particularly prone to diarrhea, which is associated with high mortality.[9,16] Husbandry and veterinary staff at giant panda breeding centers have expressed concern over a recently perceived increase in abortions and stillbirths (Loeffler and Howard, pers. comm.). Moreover, the dental abnormalities and enamel erosion that are found in many young and adult giant pandas[11,12] may be related to early infection with CDV.[2,8] The etiology of the ''Stunted Development Syndrome'' of giant pandas in China[11] also may include an infectious component.

An ultimate purpose for establishing a viable ex situ population is to release captive-born individuals into the wild. Critical to the success of such a project is an understanding of the infectious diseases in the in situ and ex situ populations and of the control and monitoring of these diseases. There is a lack of information on both the infectious disease risks to giant pandas and the prevalence of endemic diseases in the wild populations. To date, it appears that the control and monitoring of diseases has not been previously documented, and it is crucial that this information be elucidated. Hence, the goal of this study was to begin to address this need in China's ex situ population. Antibody titers were measured against CDV, CPV, CAV, CCV, canine herpesvirus (CHV), canine parainfluenza virus (CPIV), *Toxoplasma gondii*, *Neospora caninum*, and *Leptospira interrogans* in giant pandas and, for species comparison, red pandas (*Ailurus fulgens*). The results underscore the urgency for a comprehensive effort to monitor and prevent infectious diseases in these species.

MATERIALS AND METHODS

A total of 44 blood samples were collected from 19 giant pandas at the Chengdu Panda Base from 1998 through 2003. The donors were 14 females (3.5–20 yr of age) and five males (6.5–16 yr of age). Two of these individuals were from other zoos (GP 03 from Chongqing Zoo, Chongqing City, Sichuan Province; GP 11 from Beijing Zoo, Beijing City) and were temporarily maintained at the Chengdu Panda Base for breeding. Blood samples were collected opportunistically during the breeding season (February through May) in 1999, 2000, 2001, and 2003 when pandas were anesthetized for semen collection or artificial insemination. The samples obtained in 1998 were associated with the Giant Panda Biomedical Survey conducted in February and March by the International Union for The Conservation of Nature and Natural Resources (IUCN)'s Conservation Breeding Specialist Group.[10] Blood samples were centrifuged and sera stored at −20°C at the Chengdu Panda Base until analysis. Serial samples of two or more years were collected from 11 of the 19 giant pandas, resulting in the total of 44 specimens (Table 1). Samples from eight red pandas (five females, three males, aged 1 yr or 4 yr) were collected in April 2003 during routine health evaluations. Both species were anesthetized with ketamine hydrochloride (5–10 mg/kg body weight; The First Pharmacy Co., Shanghai, China) by the Chengdu Panda Base staff veterinarians.

There are no laboratories in China that routinely perform serologic assays for wildlife. Export of the serum samples to a laboratory in the United States is not permitted by Chinese authorities. Therefore, the necessary assays were established for this study in a virology laboratory at the Sichuan Agriculture University (SAU; Ya'an, Sichuan Province). The assays were first validated for giant pandas and red pandas at the New York Animal Health Diagnostic Center (Cornell University, Ithaca, New York, USA). Archived sera from four giant pandas and four red pandas of the Smithsonian's National Zoological Park in Washington, D.C., were used for assay validation. The Cornell University protocols were then used at the SAU laboratory.

Antibody titers to CDV, CAV, and CCV were measured by virus neutralization using Vero, A-72, and Madin–Darby canine kidney cells, respectively.[1,14] The antigen for the CDV assay was a wild-type CDV that had been harvested from a dog that was clinically ill with distemper in Sichuan Province. The antigens for the CAV and CCV assays were the Toronto A26/61 strain of CAV-1 (ATTC VR800) and the wild-type CCV isolated at the Baker Institute, Cornell University. The intended starting dilution for these three assays, per the Cornell University protocol, is 1:4. However, insufficient serum volume in 80% of the samples in this study necessitated higher starting dilutions of 1:6, 1:10, or 1:15 (Table 1).

Titers against CPV were measured by hemagglutination inhibition. Porcine red blood cells for the assay were acquired from a local domestic pig and prepared with Alsever's solution. The CPV-2a strain of CPV-2 (Baker Institute, Cornell University) served as the antigen.[5] The minimum sample dilution for CPV assays was 1:10.

An indirect hemagglutination inhibition assay (TPM-TEST® kit, Wampole Laboratories, Princeton, New Jersey 08540, USA) was used to measure *Toxoplasma* titers, according to the kit protocol. The minimum serum dilution for most samples was

Table 1. Results of antibody titers against canine distemper virus (CDV), canine coronavirus (CCV), canine adenovirus (CAV), canine parvovirus (CPV), canine herpesvirus (CHV), canine parainfluenza virus (CPIV), *Leptospira interrogans* (Lepto), *Neospora caninum* (Neosp), and *Toxoplasma gondii* (Toxopl) in the giant panda and red panda. The lowest serum dilution that was tested with each antigen is indicated in parentheses in the column heading. Insufficient serum volume necessitated a higher starting dilution with some samples; those starting volumes are indicated in parentheses in cells with negative results. Serum dilutions are expressed as the reciprocal.[a,c]

Animal No.	Sex	Age (yr)	Sample year	CDV (4)	CCV (4)	CAV (4)	CPV (10)	CHV (5)	CPIV (5)	Lepto (5)	Neosp (5)	Toxopl (40)
GP 01	F	3	1998	160	neg	neg	neg	neg	neg	neg	neg	neg
		4	1999	neg (20)	neg (12)	neg (24)	20	neg	neg	neg	neg	neg
		5	2000	8	neg	neg	40	neg	neg	neg	neg	neg
		6	2001	30	neg (10)	neg (20)	1,280	neg	neg	neg	neg	neg
		8	2003	4	neg	neg	640	neg	neg	neg	neg	neg
GP 02	F	7	1998	480	neg	neg	60	neg	neg	neg	neg	neg
GP 03[b]	F	19	2001	960	neg	neg	640	neg	neg	neg	neg	neg
GP 04	F	4	1998	neg (8)	neg	neg	40	neg	neg	neg	neg	neg
		5	1999	16	neg	neg	40	neg	neg	neg	neg	neg
		6	2000	neg	neg	neg	80	neg	neg	neg	neg	neg
GP 05	F	7	1999	168	neg (8)	neg (20)	640	neg	neg	neg	neg	neg
		9	2001	80	neg	neg	640	neg	neg	neg	neg	neg
GP 06	F	15	1999	80	neg	neg	768	neg	neg	neg	neg	40
		16	2000	160	neg	neg	640	neg	neg	neg	neg	80
		17	2001	60	neg (10)	neg (20)	1,280	neg	neg	neg	neg	104
		19	2003	160	neg	neg	2,560	neg	neg	neg	neg	80
GP 07	F	16	1999	480	neg (8)	neg (20)	160	neg	neg	neg	neg	80
		17	2000	480	neg	neg	640	neg	neg	neg	neg	160
		18	2001	128	neg	neg	320	neg	neg	neg	neg	neg
		20	2003	160	neg	neg	640	neg	neg	neg	neg	neg
GP 08	F	9	1999	30	neg (10)	neg (20)	640	neg	neg	neg	neg	160
		11	2001	20	neg	neg (20)	10,240	neg	neg	neg	neg	104
		13	2003	60	neg	neg	2,560	neg	neg	neg	neg	80
GP 09	F	13	1999	24	neg	neg	320	neg	neg	neg	neg	40
		14	2000	24	neg	neg	640	neg	neg	neg	neg	120
		17	2003	40	neg	neg	320	neg	neg	neg	neg	52
GP 10	F	6	1999	33	neg (10)	neg	160	neg	neg	neg	neg	neg
		7	2000	640	neg	neg	1,280	neg	neg	neg	neg	neg
		8	2001	60	neg	neg (20)	640	neg	neg	neg	neg	neg
		10	2003	48	neg	neg	640	neg	neg	neg	neg	neg
GP 11[b]	F	7	2000	neg	neg	neg	2,560	neg	neg	neg	neg	neg
GP 12	F	6	2000	320	neg	neg	640	neg	neg	neg	neg	neg
		7	2001	240	neg	neg	1,280	neg	neg	neg	neg	neg
GP 13	F	15	2000	160	neg	neg	320	neg	neg	neg	neg	160
GP 14	F	6	2003	44	neg	neg	1,280	neg	neg	neg	neg	neg
GP 15	M	6	2003	24	neg	neg	640	neg	neg	neg	neg	40
GP 16	M	11	1998	1,280	neg	neg	20	neg	neg	neg	neg	neg
		16	2003	2,560	neg	neg	80	neg	neg	neg	neg	neg
GP 17	M	16	2000	240	neg	neg	640	neg	neg	neg	neg	neg
GP 18	M	6	1998	480	neg	neg	40	neg	neg	neg	neg	40
		8	2000	480	neg (10)	neg (20)	1,280	neg	neg	neg	neg	104
		9	2001	1,280	neg	neg	1,280	neg	neg	neg	neg	80
		11	2003	1,280	neg	neg	160	neg	neg	neg	neg	80
GP 19	M	9	1998	30	neg	neg	20	neg	neg	neg	neg	80
RP 1	M	1	2003	192	neg	neg	80	neg	neg	neg	neg	neg
RP 2	F	1	2003	256	neg	neg	320	neg	neg	neg	neg	neg
RP 3	F	1	2003	1,920	neg	neg	160	neg	neg	neg	neg	neg
RP 4	F	1	2003	10,240	neg (8)	neg	40	neg	neg	neg	neg	neg
RP 5	M	4	2003	40	neg (8)	neg	80	neg	neg	neg	neg	neg
RP 6	M	4	2003	24	neg	neg	640	neg	neg	neg	neg	neg
RP 7	F	1	2003	2,560	neg	neg	80	neg	neg	neg	neg	neg
RP 8	F	1	2003	512	neg	neg	40	neg	neg	neg	neg	neg

[a] GP, giant panda; RP, red panda; F, female; M, male; neg, negative.

[b] Giant pandas on loan to Chengdu Research Base in year of sampling; unknown type and date of vaccination.

[c] Titers categorization: CDV: negative, <1:8; suspect, 1:8–1:16; positive, >1:16. CPV: negative, <1:10; suspect, 1:10–1:20; positive, >1:20. *Toxoplasma*: negative, <1:64; positive, 1:64–1:512; active titer, >1:512.

1:40; low serum volume necessitated a starting dilution of 1:52 with five of the samples (Table 1).

Giant panda serum was toxic to the indicator cells prescribed by the Cornell University protocol for the CHV and CPIV serum neutralization assays. Therefore, microdot slides were prepared to screen for the presence of serum antibodies to these two viruses by indirect fluorescent antibody (IFA) assay. A-72 cells were infected with CHV (Baker Institute, Cornell University) or CPIV (ATCC VR399) and were fixed to the slides. The percentage of infected cells on the slides was determined with antibody-positive canine sera. IFA results with the panda sera then were read relative to this positive control.

Antibody titers against *N. caninum* and five serovars of *L. interrogans* were also tested by IFA. Slides for *Leptospira* carried a cocktail of the serovars pomona, hardjo, canicola, icterohemorrhagiae/copenhageni, and grippotyposa. Reactivity of *Leptospira* slides was assessed using positive control bovine sera. *Neospora* slides were prepared according to the methods of Conrad et al.[6] Samples were screened by IFA at 1:5 and 1:50 using FITC-labeled Protein A (Zymed, San Francisco, California 94080, USA) as the secondary antibody. Binding properties of Protein A for giant panda and red panda immunoglobulins were determined using CDV-positive serum applied to Vero cells infected with CDV. All slides used for IFA assays were prepared at the Cornell laboratory and transported to the SAU laboratory.

Descriptive and exploratory statistical analyses were conducted on the 19 giant panda and eight red panda samples categorized by age and sex. The serial observations on individual pandas were treated as separate age observations. Association and interaction of antibody prevalence for CDV, CPV, and *Toxoplasma* with age, sex, year of sampling, and, for females, number of cubs, pregnancies, and duration of nursing prior to sample collection were examined with logistic predictive models under maximum likelihood estimation conditions. When multiple variables were examined, stepwise methods were used. Correlation coefficients specific for the type of binary, polychotomous, or continuous data under consideration were computed. Tests of independent means were performed after evaluation of the test results for the assumption of homogeneity of variance. The appropriate means tests then were conducted. All analyses were run on SPSS-PC version 12.0 (SPSS 12 for Windows, SPSS, Inc., 2002, Chicago, Illinois 60606 USA) and Math Cad version 11.0 (Mathsoft Engineering and Education, Inc., 2002, Cambridge, Massachusetts 02142 USA).

RESULTS

Giant pandas and red pandas at the Chengdu Panda Base are vaccinated annually between October and February with a multivalent vaccine produced for dogs against CDV, CPV, CAV, CCV, CPIV, and rabies. All but the rabies component of the vaccine were modified live viruses; and the rabies virus was killed with formalin. The vaccine is manufactured in a virology research laboratory at the Quartermaster University (Changchun, Jilin Province) in China. Although it is used throughout China in wildlife species, the manufacturers do not recommend the use of the vaccine for species other than the domestic dog. The animals in this study had been inoculated with this vaccine 3–7 mo prior to providing blood for our study. The two giant pandas on loan from other facilities (GP 03 and GP 11) were believed to have been vaccinated, but there were no records to verify dates of immunization or the source or type of vaccine.

Giant panda and red panda serum samples contained antibodies against CDV, CPV, and *Toxoplasma* (Table 1). No antibodies against CAV, CCV, CHV, CPIV, *N. caninum*, and the five-serovar cocktail of *Leptospira* were found in any of the samples (Table 1).

Antibody titers against CDV ranged from negative at 1:4 to 1:2,560 in the giant pandas and from 1:24 to 1:10,240 in the red pandas. CPV titers ranged from 1:20 to 1:10,240 in the giant pandas and from 1:40 to 1:640 in the red pandas. There was a high variability in CDV and CPV titers among individuals within each sample year and also within individuals from year to year.

CDV titers could not be predicted by any statistical model using the predictor variables of age, sex, year of sampling, or female reproductive status. Titer variance appeared to be truly random or was related to a factor that has not been identified. In contrast, CPV titers in the giant pandas were predictable on the basis of the year in which the samples were taken. Seven of eight negative titers and 29 of 35 positive titers could be predicted correctly on the basis of the year of sampling alone ($P = 0.000$), with an overall correct classification of 83.7%. The highest titers occurred in 2001, the lowest in 1998.

Six of the 19 giant pandas had antibody titers against *T. gondii*. Of the five giant pandas with serially sampled, positive titers, two sustained a positive titer over three or more years, while the others showed a decrease. *Toxoplasma gondii* titers correlated with age and CPV serostatus in females ($P = 0.000$, $R = 0.634$, overall classification of

86.8%). Older, CPV-positive females were more likely to have a positive *T. gondii* titer. All red panda samples were negative for *T. gondii*.

DISCUSSION

This study examined the potential effectiveness and risk of vaccinating giant pandas and red pandas with locally produced canine modified live virus. It also assessed the prevalence of natural exposure to certain infectious pathogens of carnivores in Chinese ex situ populations of these species. The results indicate that at least CDV, CPV, and *Toxoplasma* are of concern.

The clinical and epidemiologic significance of the pathogens or of their respective titers is unknown for giant pandas and red pandas. A low positive titer may indicate nonspecific inhibition in the assay, a waning titer from natural virus or vaccine exposure, an early sero-conversion stage, or cross-reactivity with a related virus. Serial sample analysis would be necessary to sort out this issue. As this was not possible in the present study, low positive titers were considered 'suspect.' Titers of 1:16 for CDV and 1:20 for CPV were classified as suspect. Positive titers were categorized as >1:16 for CDV and >1:20 for CPV.

In this study, five of 44 (11%) of the giant panda CDV titers were in the negative category, two of 11 (2%) were suspect, and 37 of 44 (84%) were positive. Titers in the positive category ranged from 1:40 to 1:2,560. All the red panda titers fell into the positive range (1:24 to 1:10,240). For CPV, 40 of 44 (91%) of the giant pandas' titers were in the positive range, which varied from 1:40 to 1:10,240. All of the red pandas had positive CPV titers, with a somewhat narrower range of 1:40 to 1:640. The distribution of the titers in this study showed multiple peaks, with a relatively high proportion of data points in the clinically negative range. For comparison, antibody titers against CDV in vaccinated dogs in the United States follow a normal distribution, with a median titer of 1:256. The most frequent CPV titer in this population is 1:320 (Dubovi, unpubl. data).

The high degree of variation in the CDV and CPV titers both among individuals and within individuals over time may be explained by a variation in the quality of the vaccine. The correlation of CPV vaccine titer with year of sampling supports this hypothesis. Moreover, personal communication with Chinese colleagues and personal observation (Loeffler) indicate that the manufacturing process for the vaccine does not include quality control testing. A recent report in the Chinese news revealed some of the problems with quality control of vaccines produced in that country.[4]

Variation in the quality of response to the vaccine by individual pandas also may be a possibility. The immune response to the antigens may vary among individual pandas or may be subjected to other, unknown physiologic factors. However, the degree of variation among individuals of the same species in response to a given vaccine, as was found in this study, is not the norm. Early vaccination trials in the United States with attenuated CDV resulted in consistently high antibody titers in red pandas and other sensitive species.[18] Data for vaccination with attenuated CDV in giant pandas have not been published in Western literature.

Finally, incomplete delivery of the vaccine may result in varied antibody titers. Giant pandas are vaccinated by homemade blow dart at the Chengdu Panda Base, and an injection by this method may not always be complete. Red pandas, on the other hand, are small and are physically restrained for vaccination, so that inconsistent delivery of injections seems unlikely.

Only two of five antigens in the vaccine elicited a measurable antibody response. Since there were no unvaccinated animals in the study group, one could not evaluate the possibility that the measured CDV and CPV titers were the result of natural exposure or that they had arisen in response to a combination of vaccine and naturally acquired antigen. The study of Mainka et al.[17] indicates that giant pandas are exposed to the field strains of CDV and CPV, but these two studies were conducted in areas that differ greatly in ecology and human influence and that are separated by some 150 km of mostly mountainous terrain. A recent serologic study of captive red pandas in 10 Chinese facilities indicated that those animals had been exposed to wild-type CDV and CPV, although all the titers fell into the suspect range.[19]

The lack of response to the CCV, CAV, and CPIV antigens in the vaccine again indicates an unreliable vaccine quality. Giant pandas do develop antibodies against the field strains of CAV and CCV,[17] so a lack of susceptibility to these viruses cannot explain the lack of antibody titers in this study. In the two zoos that vaccinated their red pandas in the study by Qin et al.,[19] 50% and 75% of the animals had a positive titer to CAV. One of the animals that was housed at the Chengdu Panda Base had an antibody titer of more than 1:1,024, which is consistent with the exposure of a vaccinated animal to the field virus. Vaccine titers to CCV were low in the red panda study, as they were in this study. Taken together, the data indicate that the virus strains or

their preparation for the vaccine are not appropriate for the immunization of giant pandas and red pandas.

The high CDV titers in this study were of particular concern. It has been recognized since the 1970s that modified live CDV vaccines may cause clinical disease and death in highly sensitive species such as red pandas.[3] Veterinary staff caring for red pandas in Chinese facilities have reported a high degree of morbidity and mortality following vaccination (Loeffler, unpubl. data) but continue to use this vaccine. The effect of modified live CDV vaccine in the giant pandas is unknown. Although no giant panda deaths have been directly attributable to vaccination in China, the possibility of subclinical disease may be significant. Since the virus affects epithelial cells, in utero or neonatal infection from a vaccinated dam may result in developmental effects, as seen with the giant panda Stunted Development Syndrome.[11] Vaccination of cubs with modified live virus may contribute to the gastrointestinal and respiratory illness that is so common in captive giant pandas in China.[16] The Conservation Breeding Specialist Group (CBSG) Biomedical Survey of giant pandas in China revealed an inordinately high incidence of enamel dysplasia/hypoplasia in juvenile and subadult giant pandas that were less than 4.5 yr of age.[11,16] The changes resemble those in puppies of domestic dogs exposed to CDV neonatally. These puppies develop multiple and permanent dental abnormalities affecting both the enamel and dentine.[2,8]

The data from this study reemphasize the importance of using extreme caution in the administration of vaccines that are not specific for a particular species. This is particularly critical for species about which so little is understood in terms of infectious disease susceptibility.

A recombinant CDV vaccine (Purevax®, Merial, Athens, Georgia 30601, USA) is available for use in the domestic ferret (*Mustela putorius*), a species that is also highly sensitive to CDV. The vaccine is safe and effective in ferrets, and clinical trials were therefore conducted in giant pandas and red pandas in the United States. To date, it has elicited positive antibody titers (1:16 to 1:512) with no untoward effects in either of these two species (Montali, unpubl. data). It is recommended that this vaccine be used in China for giant pandas and red pandas so that they are protected at least against canine distemper.

Toxoplasma gondii titers were classified according to the Cornell University protocol for domestic species tested with the TPM-TEST® kit. Titers below 1:64 were considered negative, while titers from 1:64 to 1:512 were considered positive and indicated previous exposure. The positive titers in the seven giant pandas were of a magnitude that indicates previous, but not necessarily recent, exposure without active clinical disease.

Toxoplasma infections appear to have a localized distribution in Chinese captive facilities. The recent red panda study found up to 94% of the animals at four of the 10 institutions to be seropositive, with 52% of titers in the range associated with clinical disease in domestic species (>1:512).[19] Toxoplasmosis is therefore a potential concern in giant pandas and red pandas in China, although the clinical implications of *T. gondii* infection in these species are unknown.

On the premises that the domestic cat is the common definitive host for *T. gondii* in urban and suburban China and that a variety of small mammals and birds serve as indirect or intermediate hosts,[7] the most likely sources of infection for pandas are ingestion of oocytes by direct contact with cat feces or of tachyzoites from eating infected rodents or birds. Feral domestic cats and rodents are ubiquitous in captive wildlife facilities in China and usually gain easy access to enclosures. It is interesting that all seroconversions from a titer less than 1:64 to higher than 1:64 in this study occurred before 2000. Recent improvements to the security of the giant panda yards at the Chengdu Panda Base and a campaign to eradicate feral cats on the property may explain the cessation of seroconversions.

The positive correlation of *Toxoplasma* titers with CPV in the giant pandas indicates a possible interrelationship that deserves further investigation. This correlation may indicate changes in the immune activity of the giant pandas with one or the other pathogen. Alternatively, it may simply indicate a temporal (epidemiologic) phase in the history of these animals at the Chengdu Panda Base.

One of the practical and important benefits of the present study is that sample analyses were conducted in China as part of our long-term commitment to capacity building.[21] Increasing regulations make it prohibitively expensive and time-consuming to consider biomaterials export for analysis in Western countries. Sample export also preempts the training necessary for China's emerging generation of conservation biologists and animal health scientists to become self-sufficient in protecting their own biodiversity. The development of a service animal pathogen diagnostics laboratory (or laboratories) in China is critical for the study and monitoring of infectious pathogens. The recent epidemics of SARS[15] and avian influenza virus, with their im-

plications for wildlife,[13] emphasize the urgent necessity of such efforts.

A high priority in this context is the development of a larger data set that includes as many Chinese panda institutions as possible. Each facility tends to have different husbandry and veterinary care practices. Opportunities for exposure to heterospecifics and potentially panzootic diseases also differ among institutions. Samples from unvaccinated pandas will help to determine the level and type of natural disease exposure in captive populations. Longitudinal monitoring is necessary to understand the epidemiology of infectious pathogens relevant to these species.

Given the vulnerability of giant panda and red panda populations, intensive investigation of infectious diseases in these species is warranted. The resulting information is needed to effectively protect both the ex situ and in situ populations. Those pathogens against which antibody titers in the present study were negative (i.e., *L. interrogans*) should also be examined in order to ensure that the negative results were not due to a difference between the laboratory strains and Chinese field strains of the antigens. There are approximately 200 serovars that cause disease, and very little is known about *Leptospira* in animals in China. In this study, the five serovars most commonly infecting dogs in the United States were examined, but these may not be the most pertinent for giant pandas and red pandas in China. Leptospirosis may be a disease of special concern for the giant panda, because ex situ facilities have high populations of feral rodents. Previous histopathologic examinations of kidney sections from four giant pandas that died at the Beijing Zoo indicated that leptospirosis was high on the differential diagnoses list in each of these cases, but there were no assays available in China to confirm the diagnosis (Montali, unpubl. data).

In summary, further research is needed to understand the role of infectious diseases in giant pandas and red pandas. The findings of this study support the urgency for more detailed studies that include annual monitoring, safe preventive health measures, and appropriate veterinary management. The highest priority for achieving this goal is the establishment of a wildlife disease diagnostic laboratory with trained specialists in China. This resource would be invaluable to China's rapidly growing efforts to preserve its unique biodiversity.

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