SEROSURVEY OF INFECTIOUS DISEASE AGENTS OF CARNIVORES IN CAPTIVE RED PANDAS (AILURUS FULGENS) IN CHINA

Qin Qin, M.S., Fuwen Wei, Ph.D., Ming Li, Ph.D., Edward J. Dubovi, Ph.D., and I. Kati Loeffler, D.V.M., Ph.D.

Abstract: The future of the endangered red panda (Ailurus fulgens) depends in part on the development of protective measures against infectious diseases. The present study is a first step toward improved understanding of infectious diseases in the species’ home regions. Serum samples obtained from 73 red pandas in 10 captive facilities in southwest, east, and northeast China from October to December 2004 were tested for antibodies against nine common infectious pathogens of carnivores. Antibody titers against canine distemper virus (CDV), canine parvovirus (CPV), and canine adenovirus (CAV) in the three facilities in which red pandas were vaccinated were highly variable. The CAV titer in one vaccinated red panda was high enough to suggest infection with the field virus following vaccination. Together with anecdotal reports of vaccine-associated morbidity and mortality, our results suggest that the Chinese vaccine is not suitable for this species. In the seven unvaccinated groups, CDV titers were low and occurred in 20–100% of the animals; antibody titers against CPV were found in seven of eight areas. Only one of 61 and two of 61 unvaccinated red pandas had CAV and canine coronavirus titers, respectively, and these titers were all low. Positive titers to Toxoplasma gondii were found in four locations (33–94% seropositive); the titers in 52% of seropositive individuals were of a magnitude consistent with active disease in other species (1:1,024 to ≥1:4,096). One red panda in each of three locations was seropositive for Neospora caninum. Antibodies against canine herpesvirus and Brucella canis were not detected in any of the samples. Only one of the 73 red pandas had a weak positive influenza A titer. The results of this study emphasize the need for research on and protection against infectious diseases of red pandas and other endangered species in China.

Key words: Red panda, Ailurus fulgens, serosurvey, infectious diseases, China.

INTRODUCTION

The red panda (Ailurus fulgens) is an endangered species of the order Procyonidae. It is native to the Himalayas and southwestern China. The species is listed on the International Union for the Conservation of Nature and Natural Resources (IUCN) Red List as endangered and in Convention on International Traffic in Endangered Species (CITES) Appendix I. It has Class 2 protection status in China, where its population continues to decline because of habitat loss and fragmentation, and poaching for the captive wildlife and fur trades.

The impact of infectious disease on wildlife populations has been documented in a variety of wildlife species and has highlighted the need to consider infectious disease in conservation plans. Red pandas are known to be highly susceptible to canine distemper virus (CDV), but otherwise very little information exists about infectious diseases in this species, particularly in regions to which it is native. These areas generally lack quarantine procedures, disease monitoring, and vaccination practices for captive wildlife and livestock despite a high movement of animals among captive wildlife facilities. The rapid rise in populations of domestic carnivores in these regions increases the potential for exposure of captive and free-ranging wildlife to their diseases and enhances conditions for the emergence of new pathogens. Together, these factors present the potential for significant degradation of populations of red panda (and other endangered species) by infectious disease.

The aim of this study was to investigate the exposure of red pandas in Chinese captive facilities to common infectious pathogens of carnivores, and to evaluate their antibody responses to locally produced vaccines.

MATERIALS AND METHODS

Sample collection

Blood samples were obtained from 73 (29 males, 44 females) red pandas in 10 captive facilities in China from October to December 2004. This is the period between weaning of cubs and the onset of the next breeding season, and was selected to cause...
Figure 1. Locations of study sites in mainland China. BJ: Beijing Zoo; AN: Hefei Zoo and Balihe Scenic Area Wildlife Park; FZ: Fuzhou Giant Panda Breeding Center; YN: Kunming Wildlife Park; YL: Yele Nature Reserve; CD: Chengdu Research Base of Giant Panda Breeding and Chengdu Zoo; CQ: Chongqing Wildlife Park and Chongqing Zoo.

The red pandas at the YL were kept in a small facility for injured or orphaned animals found in the reserve. The CQZ red pandas from which samples were obtained were located at the breeding facility 10 km outside of the city on a mountainside; these animals are rotated through the exhibit in the zoo, which is located within the city. The CPB and FZ are breeding centers for giant pandas and red pandas but are open to visitors year round. Relocation of red pandas from one facility to another or from the wild into captivity is done without quarantine or other disease control procedures.

Ten of the red pandas in the study had been born in captivity, 50 had been captured from the wild, and the origin of 13 was unknown. With the exception of the five red pandas at FZ, which had arrived at the facility 9 days prior to sample collection, all those caught from the wild or of unknown origin were reported to have been in captivity for at least 3 mo. However, no written records had been maintained for this population. It is unknown when the FZ animals had been captured.

Age estimates were based on verbal accounts of the red panda keepers and examination of teeth and general condition of the animals by the research team, and ranged from 1 yr to more than 8 yr. Dental estimates were as follows: no or slight wear and staining was rated as young (up to 3 yr), moderate wear and staining was rated as middle-aged (4–7 yr), and severe wear and staining were rated as old (8 yr and older).

Both the CDZ and CPB had vaccinated their red pandas 6–9 mo prior to blood sample collection; vaccination was by i.m. injection with a Chinese-manufactured modified live vaccine against CDV, canine parvovirus (CPV), canine adenovirus (CAV), canine coronavirus (CCV), canine parainfluenza virus, and rabies virus. BJ had vaccinated with an inactivated CDV vaccine 2 mo before sample collection. The strain, dose, and concentration of virus in the vaccines were unknown, or at least unobtainable. At the time of the most recent vaccination, age of the animals ranged from 3 mo to more than 8 yr.

At the time of blood sample collection, most of the red pandas appeared to be clinically healthy and
Serum samples were stored at −20°C, and then centrifuged at 15,000 g.

Blood was collected from the cephalic or saphenous veins into a disposable 5-ml syringe (Fisher Healthcare, Swedensboro, New Jersey 08085, USA), transferred to 5-ml glass tubes (Vacutainer, Kynoch Healthcare, Birmingham, England); or with “846” (acetyl-promazine, xylazine, morphine; individual drug concentrations proprietary and unobtainable; Institute of Veterinary Medicine, Changchun, China); or by a stressed mother when they were still in the nest. The fur on the hind limbs of a yearling male sampled at the CPB had had their ears chewed off, either in a fight with another red panda or by a stressed mother when they were still in the nest.

Antigens in these assays were the Ondesterpoort trimer and a serum dilution of 1:4 was used to screen sera from 73 red pandas from 10 different captive facilities (HF, CPB, CQWP, FY, and CZ) administered all anesthesia and oversaw all sampling procedures. Red pandas were immobilized with ketamine (First Pharmacy Co., Shanghai, China) with or without diazepam (Xudong Pharmacy Co., Shanghai, China); with “846” (acetyl-promazine, xylazine, morphine; individual drug concentrations proprietary and unobtainable; Institute of Veterinary Medicine, Changchun, China); or were manually restrained. Three to five milliliters of blood were collected from the cephalic or saphenous veins into a disposable 5-ml syringe (Fisher Healthcare, Swedensboro, New Jersey 08085, USA), transferred to 5-ml glass tubes (Vacutainer, Beckton Dickinson, Franklin Lakes, New Jersey 07417, USA), stored on ice for 4–24 hr postcollection, and then centrifuged at 15,000 g for 15 min. Serum samples were stored at −70°C until analysis.

Serologic analysis
Serologic assays were performed at the Animal Health Diagnostic Center at Cornell University (Ithaca, New York, USA). All 73 serum samples were assayed for the presence of antibodies against CDV, infectious canine hepatitis virus (CAV-1), CCV, and canine herpesvirus (CHV) by virus neutralization.1,17 Indicator cells for each assay were Vero, Madin–Darby canine kidney (MDCK), A-72, and SV-40 canine skin cells, respectively. Antigens in these assays were the Ondesterpoort strain of CDV, the Baker Institute strain of CAV-1, the wild-type CCV isolated at Cornell University, and the F205 strain of CHV. Serum dilutions began at 1:8 and were reported as the last dilution at which no cytopathic effect was observed in indicator cells. Antibody titers against CPV-2 were measured by hemagglutination inhibition with the use of porcine red blood cells and the CPV-2a strain of CPV,7 starting with serum dilutions of 1:10. Sera were tested for Toxoplasma gondii antibodies with the Toxoplasma TPM-TEST indirect hemagglutination test kit (Wampole Laboratories, Cranbury, New Jersey, USA) at starting dilutions of 1:32 and continuing twofold through 1:4,096. The presence of antibodies against influenza A viruses was detected with an agar gel immunodiffusion (AGID) test that indicates reactivity to the nucleoprotein (NP).2 Serum samples were used undiluted, and reactivity was recorded at 24, 48, and 72 hr. Sera were screened at 1:20 and 1:40 for antibodies against Neospora caninum by indirect fluorescent antibody (IFA) assay,8 using FITC-labeled goat anti-raccoon secondary antibody (Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA). The reactivity of anti-raccoon serum with red panda serum was confirmed using CDV-infected cells and a positive-CDV red panda serum. Rapid slide agglutination using the Brucella canis M-strain antigen and a serum dilution of 1:4 was used to screen for antibodies to B. canis.9

Statistics
Statistical analysis was performed with SPSS version 10.0 software (SPSS Inc., Chicago, Illinois, USA). Chi-square and likelihood ratio tests were used to compare antibody titers in vaccinated and unvaccinated animals for CDV, CPV, and CAV. A Student’s t-test was used for CCAV analysis. Variation of CDV, CPV, and CAV vaccine titers with age and sex was assessed with the Mann–Whitney U-test. To determine if there was an association between Toxoplasma titers with age and/or sex, a Kruskal–Wallis test was utilized.

RESULTS AND DISCUSSION
Sera from 73 red pandas from 10 different captive facilities in China were tested for antibodies against CDV, CPV-2, CAV-1, CCV, CHV, T. gondii, N. canis, B. canis, and influenza A virus (Table 1). It is not known at what level antibody titers to these pathogens are significant in red pandas, nor at what level they are protective. A low positive titer may suggest nonspecific inhibition in the assay, a waning titer from exposure to the virus (or vaccine) some time ago, an early stage in seroconversion, or cross-reactivity to a related virus. Serial samples and virus isolates from the test species would be necessary to determine which of these factors

in good nutritional condition. Two adult females in the sampled group at the CQWP were overweight. An adult male and an old female sampled at the CQZ were very severely underweight, and the red pandas at that particular facility, especially the cubs, had a high incidence of sarcoptic mange. One young male at YL was also underweight and the submandibular lymph nodes of an old male at that same facility were enlarged, most likely related to dental disease. Those at the CPB were crowded in their enclosure and fought a great deal; several animals in that group had mutilated tails and ears, and one yearling male that was sampled had fresh bite wounds. An adult male sampled at the CQWP and a yearling male sampled at the CPB had had their ears chewed off, either in a fight with another red panda or by a stressed mother when they were still in the nest. The fur on the hind limbs of a yearling male sampled at the CPB was badly matted, with what appeared to be a fungal infection present on the skin beneath the mats.

A veterinarian from BJ and/or from one of the participating facilities (HF, CPB, CQWP, FY, and CZ) administered all anesthesia and oversaw all sampling procedures. Red pandas were immobilized with ketamine (First Pharmacy Co., Shanghai, China) with or without diazepam (Xudong Pharmacy Co., Shanghai, China); with “846” (acetyl-promazine, xylazine, morphine; individual drug concentrations proprietary and unobtainable; Institute of Veterinary Medicine, Changchun, China); or were manually restrained. Three to five milliliters of blood were collected from the cephalic or saphenous veins into a disposable 5-ml syringe (Fisher Healthcare, Swedensboro, New Jersey 08085, USA), transferred to 5-ml glass tubes (Vacutainer, Beckton Dickinson, Franklin Lakes, New Jersey 07417, USA), stored on ice for 4–24 hr postcollection, and then centrifuged at 15,000 g for 15 min. Serum samples were stored at −70°C until analysis.

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Table 1. Number and percentage (in parentheses) of red panda serum samples with negative, ‘suspect,’ or positive antibody titers (titer range indicated for each pathogen) against canine distemper virus (CDV), canine parvovirus (CPV), canine adenovirus (CAV), canine coronavirus (CCV), canine herpesvirus (CHV), *Toxoplasma gondii, Neospora caninum, Brucella canis* and influenza A in each study location: Hefei Zoo (HF), Balihe Scenic Area Wildlife Park (FY), Yele Nature Reserve (YL), Chengdu Research Base of Giant Panda Breeding (CPB), Chengdu Zoo (CDZ), Chongqing Wildlife Park (CQWP), Chongqing Zoo (CQZ), Kunming Wildlife Park (YN), Fuzhou Giant Panda Breeding Center in Fujian Province (FZ), and Beijing Zoo (BJ).

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<th>CPB</th>
<th>CDZ</th>
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<td>Influenza A</td>
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<tr>
<td>Negative at 48 hr</td>
<td>6 (100)</td>
<td>6 (100)</td>
<td>5 (100)</td>
<td>8 (100)</td>
<td>4 (100)</td>
<td>16 (100)</td>
<td>10 (100)</td>
<td>8 (100)</td>
<td>5 (100)</td>
<td>4 (80)</td>
<td></td>
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<tr>
<td>Positive at 48 hr</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (20)</td>
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* Vaccinated with modified live CDV, CCV, CPV-2, CPV, CAV-1, and rabies.

** Vaccinated with modified live CDV.
would be the likely explanation. Unfortunately, this determination was not possible in the present study. A conservative approach to interpretation of the data is to consider low positive titers as “suspect.” In this study, antibody titers were classified as negative (negative at the lowest test dilution), suspect (low positive), and positive (Table 1).

**Red pandas vaccinated with the Chinese canine vaccine**

Two groups of red pandas (CPB and CDZ; 12 total) had been inoculated 6–9 mo prior to the sample date with a multivalent modified live vaccine that is produced for dogs in China. One group of five red pandas (BJ) had been vaccinated with a Chinese monovalent inactivated CDV vaccine 2 mo before sample collection. Antibody titers against CDV \( (P = 0.000) \), CPV \( (P = 0.000) \), and CAV \( (P = 0.000) \) in this study were higher in vaccinated groups than in unvaccinated groups. The CCV vaccine used in these animals elicited no measurable antibody response in any but one individual, whose titer was at the lowest limit of detection and hence unlikely to bear significance.

Vaccine titers to CDV, CAV, and CPV varied from negative to high positive (Table 1; Fig. 2). Titers against CDV and CAV ranged from negative at 1:8 (1/17 and 2/12 individuals, respectively) to suspect (1:8 to 1:16; 1/17 and 2/12, respectively) to positive (>1:16; CDV: 16/17 with titers up to 1:512; CAV: 8/12 with titers up to 1:1,024; Table 1; Fig. 2a, b). CPV titers in vaccinated red pandas ranged from positive (16/17) at 1:10 to 1:320, with two of 12 in the suspect range below 1:40 (Table 1; Fig. 2c). In vaccinated dogs in the United States, the most frequent CPV titer is 1:640, and the mean CDV titer is 1:256, with the distribution of values as a normal bell-shaped curve (Dubovi, unpubl. data). Clearly, the magnitude and distribution of the values in the vaccinated red pandas in this study do not meet the expectations with an effective vaccine.

A similar variability in vaccine titers was found in another study of giant pandas and red pandas in a Chinese giant panda breeding facility (Loeffler, unpubl. data). The high variability of the vaccine response among individuals and the consistency of this variability in different facilities suggest that the quality of the vaccine (including the antigenicity and concentration of antigen) is inconsistent. There is little quality control in the production of many vaccines in China, particularly for those produced in university or smaller private laboratories. The vaccines used in the red pandas in this study were produced under these conditions. A recent story in the Chinese press revealed the issue of substandard vaccine production in China. Alternative explanations to the poor vaccine response include inconsistent delivery of the vaccine or a variability in response of the individual red pandas to the antigens. Neither of these is likely, as red pandas generally weigh less than 6 kg and are physically re-
strained for vaccination in China. In addition, vaccine responses to these same antigens in American and European zoos follow predictable patterns.21

The CAV titer of one vaccinated red panda at CPB was high enough to suggest exposure of a vaccinated animal to a field strain of the virus (≥1:1,024). The domestic dog population in China carries CAV endemically (Loeffler, unpubl. data) and could easily serve as a source of virus to captive wildlife. This red panda was not reported to have shown any signs of illness recently, but its husbandry among a large group of red pandas was such that a reclusive individual could easily be overlooked. The same is the case for red pandas vaccinated with the Chinese modified live CDV vaccines. Mortality in red pandas inoculated with these vaccines can be as high as 100%,3 and anecdotal reports of vaccine-associated disease and mortality in red pandas in China are common.

Vaccine titers in this study did not vary with age or sex. It is possible that they may vary with the number of successive years over which the animals had been vaccinated, but serial samples or past medical records were not available for the necessary analysis.

Antibody titers in unvaccinated red pandas

CDV is a highly contagious morbillivirus that causes multisystemic disease in a variety of domestic and nondomestic carnivore species. Mortality may be very high in some species, e.g., ferrets and red pandas. All nonnegative CDV titers in the seven unvaccinated locations in the present study fell into the “suspect” category (Table 1; Fig. 2a). This implies some degree of natural exposure to CDV, but the relatively low antibody titers in this species, which is so susceptible to disease caused by CDV, is an interesting finding. One explanation may be that the survival rate of infected animals is low and exposed animals do not exist in the sampled population. Another possible explanation might be that the field virus does not elicit a strong immune response even though it kills the infected animal. Also, as indicated previously, the assay may not be sufficiently sensitive. Because we do not have the field strain of CDV that is circulating in Chinese red pandas, we are unable to compare the antigenic similarity between the field strain and the virus that was used in these neutralization assays.

CPV is a rapidly emerging virus that affects an increasing number of domestic and wild carnivores.24 It attacks rapidly dividing cells and results in gastroenteritis, myocarditis (in canids), and compromised fetal development (in felids). More than half (31/61) of unvaccinated red pandas in seven of eight study areas had CPV titers (Table 1; Figs. 2c, 3). With the exception of one titer at 1:160 and three in each of three different locations at 1:40, these titers were also all in the suspect range (≤1:20; Figs. 2c, 3). Interestingly, two of the red pandas with titers of 1:10 (one from BJ, one from YN) and one with a titer of 1:160 (BJ) tested CPV-positive on a polymerase chain reaction screen of rectal swabs. A novel red panda parvovirus was isolated from the YN sample.22 Fecal shedding of CPV generally occurs only during the acute phase of the infection, which would suggest that these three red pandas, particularly the YN animal, had only recently been infected. The authors learned that several of the red pandas at YN became ill and died a few weeks after the sample date but a disease diagnosis or cause of death were not determined. Possible explanations for the low CPV titers in unvaccinated red pandas in this study include the following: that the antibody response in this species is poor or short-lived following virus exposure, that natural exposure of captive red pandas may be uncommon in China, that the antigen used in this study cross-reacts poorly with a red panda parvovirus, or that infection with this virus results in high mortality rates and causes a sampling bias.

Infectious hepatitis caused by CAV-1 has been found in canids, bears, and skunks.26 CCV causes enteritis in canids. Mortality is generally low except in very young animals, but can rise when compounded with secondary bacterial infections. Antibody titers against CAV and CCV in unvaccinated red pandas were rare in this study. Only one of 61 and two of 61 unvaccinated red pandas had CAV

![Figure 3. Distribution of CPV antibody titers in unvaccinated, captive red pandas in eight Chinese facilities.](https://bioone.org/journals/Journal-of-Zoo-and-Wildlife-Medicine on 15 Sep 2020 Terms of Use: https://bioone.org/terms-of-use)
Figure 4. Percentage of negative (<1:64), low positive (1:64 to 1:128), intermediate (1:256 to 1:512), and active (1:1,024 to ≥1:4,096) T. gondii titers in the four locations in which red pandas had positive titers.

...and CCV titers, respectively, and these were in the suspect range (1:8 to 1:16).

It is difficult to interpret the significance of the suspect titers without analysis of a duplicate sample drawn some 2–3 wk after the initial one. Unfortunately, political restrictions precluded the possibility of doing this. The low titers (CDV, CPV, CAV, CCV) and low frequency of positive titers (CAV, CCV) in unvaccinated red pandas are interesting in the context of the endemic prevalence of these pathogens in dogs and cats in China (Loeffler, pers. obs.) and, in the case of CDV and CPV, in a number of wildlife species throughout the world. The low incidence and magnitude of CCV titers in this study may be explained in several ways, including that red pandas may mount a low antibody response to the Chinese canine vaccine, that natural exposure of captive red pandas in China may be low, or that the coronavirus of red pandas may be antigenically different from that of dogs such that the serum neutralization assay used in this study produces false negative results in unvaccinated individuals. Lack of natural exposure and/or poor assay sensitivity may also explain the complete lack of CHV and B. canis titers in any of the red pandas in this study (Table 1).

Serologic assay of influenza A infection was based on the detection of antibodies to the NP protein. Influenza A viruses have been found in many domestic and wildlife species and are an important focus of research on emergent diseases. Only one of the red pandas in this study showed a positive assay result, which suggested either that influenza A infection is not prevalent in the study populations or that the assay is not sensitive with red panda sera.

Antibody titers against the protozoan parasites T. gondii and N. caninum

Toxoplasma gondii infections have been found in a variety of wildlife, including mustelids, procyonids, ursids, felids, canids, and marsupials. Infection may cause multisystemic disease, particularly in young animals, and can result in abortion and stillbirth. Seropositive red pandas ranged from 33% to 94% in four of the 10 facilities in this study. Among the seropositive individuals, 52% had T. gondii titers of a magnitude consistent with clinical disease in domestic species (Table 1; Fig. 4). Titers appeared higher in adults than in young animals but this result was not statistically significant (P = 0.373, Kruskal–Wallis test). Again, staff at the four facilities had not observed clinical disease other than occasional diarrhea and respiratory signs. These animals were reported to be rarely ill. However, staff at the CQZ later noted that a significant percentage of red panda cubs are lost to sarcoptic mange each year, and one adult red panda there was being treated for it at the time of sampling for this study.

Reproduction in red pandas is generally poor in Chinese captive facilities and appears to be primarily because of inappropriate husbandry and, in hand-reared young, failure of passive transfer and malnutrition (Loeffler, unpubl. data). Abortion and stillbirth, as a possible consequence of infection...
with *T. gondii* or other pathogens, could be easily overlooked, because female red pandas usually consume expelled fetuses and unift neonates. In keeping with the current understanding that the only definitive host for *T. gondii* is the cat and the obligatory intermediate host is the rodent, the red pandas must be ingesting oocysts shed by cats who defecate in the enclosures, or they are eating infected mice. Feral cats are ubiquitous in Chinese cities and generally have easy access to the animal enclosures. The clinical significance of *T. gondii* infections in captive red pandas remains to be investigated.

*Neospora caninum* is recognized as a cause of encephalomyelitis in dogs, which have been identified as a definitive host of *N. caninum*, whereas cats and a variety of hoof stock are natural intermediate hosts. Three of the red panda samples tested positive in the *N. caninum* IFA screen in this study (two at 1:40 and one ≥1:80; Table 1). These data present sufficient evidence to warrant further investigation of the risk and significance of *N. caninum* infection among captive red pandas in Chinese facilities.

In conclusion, the results of this study raised a number of questions regarding the prevalence, risk, and significance of carnivore infectious diseases in captive red pandas in China. Moreover, they emphasize the need for research and for the importance of developing means by which to protect these species from infectious disease.

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**LITERATURE CITED**


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