Serosurvey of selected viruses in captive giant pandas (*Ailuropoda melanoleuca*) in China

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ABSTRACT

Serum samples from 92 giant pandas in three captive facilities were tested for antibodies against five viruses of carnivores. Antibody titers against canine distemper virus (CDV) in two facilities in which giant pandas were vaccinated were variable. The canine adenovirus (CAV-1) and canine parvovirus (CPV) titers in vaccinated group were both positive, but titers were not high and varied among individual except one vaccinated panda had extremely high CAV-1 titer, indicating infection with the field virus following vaccination. Our results suggest that the vaccines used for these giant pandas do not elicit consistent antibody titers. Antibody titers against CDV, CPV and CAV-1 in unvaccinated giant pandas were highly variable, especially CPV titer. Almost half of sera were CPV antibody positive, and CPV titers were high enough to suggest infection with the virus. Canine coronavirus (CCV) and canine parainfluenza virus (CPIV) titers were not detected in all serum samples. The results of this study emphasize the need for research on infectious diseases of giant pandas and development of suitable vaccines for the species.

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1. Introduction

The giant panda (*Ailuropoda melanoleuca*) is endemic to China and is listed in the International Union for the Conservation of Nature (IUCN) as endangered and in Conservation on International Traffic in Endangered Species (CITES) Appendix I. It has Class 1 protection status in China, where its population in the wild is estimated to be 1600 individuals and the captive population is more than 250 (Wei et al., 2006). In the past decades, much consideration focused on the nutrition and reproduction of captive giant pandas, while prevention and control of infectious diseases were ignored. 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 (Dobson and Foufopoulos, 2001).

Captive giant pandas in China are kept in zoos or breeding centers, where other animals, such as the red panda, are also kept; and some visitors can contact with giant pandas closely to take photos, and even feed to animals, although it is forbidden. Under these circumstances, it can not be avoided that pathogens could transmit among species and individuals including giant pandas, and that visitors can also transmit pathogens to animals. Although dogs and cats are not allowed to enter the place where giant pandas live, breeders or visitors who have pets probably transmit pathogens to pandas.

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It has been reported that canine distemper virus (CDV), canine parvovirus (CPV), and canine coronavirus (CCV) have been implicated in disease and mortality in giant pandas (Qiu and Mainka, 1993; Mainka et al., 1994; Qiao et al., 2004). For instance, three pandas were infected with CDV, of which two died in Chongqing Zoo, and two pandas died of CDV in Nanjing Zoo (Hu et al., 1997; Huang, 1983). Giant pandas with CPV infection showed diarrhea, vomiting and water-like feces (Wu et al., 1988), and CCV caused acute enteritis of pandas in Fuzhou Zoo in 1987. CDV and CCV strains were also isolated from giant pandas (Li et al., 1999; Hu et al., 2004). Mainka et al. (1994) assayed antibody titers against CDV, CCV, canine herpesvirus (CHV), pseudorabies virus (PRV), canine adenovirus type 2 (CAV-2), and CPV in sera from eight unvaccinated pandas, and detected antibodies against CDV, CCV, CAV-2 and CPV. Loeffler et al. (2007) carried out serologic analysis of 19 giant pandas at Chengdu Research Base of Giant Panda Breeding (CPB) who were vaccinated with a common dog vaccine, and detected antibodies against CDV, CPV and *Toxoplasma* that varied among individuals and from year to year in and among individuals.

In China, however, there is no standard vaccine strategy for captive giant pandas. For example, most giant pandas at China Conservation and Research Center for the Giant Panda (Wolong Research Center, WL) are not vaccinated, while the CPB used live multivalent vaccines (including CDV, CPV, CAV-1, CCV, Canine parainfluenza virus (CPIV), and rabies virus) for dogs, and the Beijing Zoo (BJZ) used killed CDV vaccine made for dogs. Although no giant panda deaths have been directly attributable to attenuated live virus vaccines in China, the possibility of subclinical disease may be significant. Neonatal infection from a vaccinated dam may result in the giant panda Stunted Development Syndrome (Janssen et al., 2006). Vaccination of cubs with modified live CDV vaccines may contribute to the gastrointestinal and respiratory illness. What's more, the dental abnormalities and enamel erosion that are found in giant pandas may be related to early infection with CDV (Bittegeko et al., 1995; Janssen et al., 2006).

The objectives of this study were to investigate the exposure of giant pandas in Chinese captive facilities to common infectious viruses of carnivores, to compare the difference in antibody titers between vaccinated and unvaccinated giant pandas, and to evaluate their antibody responses to locally produced vaccines.

2. Materials and methods

2.1. Sample collection

Serum samples were obtained from 92 (31 males, 60 females, 1 unknown gender) giant panda individuals in three captive facilities in China from 1994 to 2005. Sixty-seven samples were from WL; 20 were from CPB and five were from BJZ (Tables 1 and 2). Blood samples were collected when pandas were anesthetized for semen collection, artificial insemination or routine physical examination during February to June. Both the CPB and BJZ had vaccinated their pandas 4–5 months prior to blood sample collection. Pandas were anesthetized with keta-

Table 1
Number of giant panda serum samples per year and facility.

Location	1994	2000	2001	2002	2003	2004	2005
WL	1	2	5	13	11	15	20
CPB	0	0	0	0	5	9	6
BJZ	0	0	0	0	0	2	3
Total	1	2	5	13	16	26	29

WL: China Conservation and Research Center for the Giant Panda (Wolong Research Center); CPB: Chengdu Research Base of Giant Panda Breeding; BJZ: Beijing Zoo.

Table 2
Number of samples for different sex and age of giant pandas in each location.

Location	Number of samples					Total
	Female	Male	Young[a]	Adult	Old	
WL	46	21	34	21	12	67
CPB	11	8	2	11	6	20 (1 unknown)
BJZ	3	2	1	4	0	5
Total	60	31	37	37	18	92 (1 unknown)

[a] Range of young, adult and old was defined as: young: <4.5 years of age; adult: 5–18 years of age; old: >18 years of age.

mine hydrochloride (5–10 mg/kg body weight; The First Pharmacy Co., Shanghai, China) by staff veterinarians. CPB had vaccinated their giant pandas with a Chinese manufactured multivalent vaccine against CDV, CPV, CAV-1, CCV, canine parainfluenza virus (CPIV) and rabies virus for dogs. All but the rabies component of the vaccine were modified live viruses; and the rabies virus was killed with formalin. BJZ had vaccinated with an inactivated CDV vaccine. The strain, dose, and concentration of virus in the vaccines were unknown, or at least unobtainable. One giant panda called Dadi was born in WL, and then transferred to BJZ in October, 2004 and was vaccinated with inactive CDV vaccine. We collected a serum sample from Dadi while he was at WL in April 2004 and then at BJZ in March 2005. Serum samples were stored at −20 °C until analysis.

2.2. Serologic analysis

All 92 serum samples were assayed for the presence of antibodies against CDV, CAV-1 and CCV by virus neutralization (Appel and Robson, 1973; Kimber et al., 2000). Indicator cells for each assay were Vero, Madin-Darby canine kidney (MDCK), A-72 cells, respectively (cell lines were obtained from the China Institute of Veterinary Drug Control). Serum dilutions began at 1:8 and are reported as the last dilution at which no cytopathic effect was observed in indicator cells. Antibody titers against CPV-2 and CPIV were measured by hemagglutination inhibition with the use of porcine red blood cells and chicken red blood cells, respectively, starting with serum dilutions of 1:10. Hemagglutination inhibition assays were performed according to the method described by Carmichael et al. (1980). Results are reported as the highest dilution at which no hemagglutination was observed. Antigens used

in all of these assays were the virus strains isolated from dogs at the Veterinary Research Institute, the Academy of Military Medical Sciences. Positive controls were sera from dogs that were confirmed be infected with these viruses.

2.3. Statistics

Statistical analysis was performed with SPSS version 11.0 software (SPSS Inc., Chicago, IL, USA). Mann–Whitney test was used to compare antibody titers between vaccinated and unvaccinated animals for CDV, CPV and CAV-1. Variations of CDV, CPV and CAV-1 vaccine titers with age and sex were assessed with the Kruskal–Wallis and Mann–Whitney tests, respectively.

3. Results

Ninety-two serum samples from giant pandas from three captive facilities were tested for antibodies against CDV, CAV-1, CCV, CPV-2 and CPIV (Table 3). In this study, antibody titers were classified as negative (negative at the lowest test dilution), suspect (low positive), and positive. Titers up to 1:16 for CDV and CAV-1 and 1:20 for CPV were classified as suspect. Positive titers were categorized as >1:16 for CDV and CAV-1 and >1:20 for CPV.

3.1. Antibody titers in vaccinated giant pandas

Vaccine titers to CDV, CPV and CAV-1 varied from negative to high positive (Table 3, Fig. 1). Titers against CDV ranged from negative at 1:8 (12/25) to suspect (1:8 to 1:16, 6/25) to positive (1:16 to 1:256, 7/25; Table 3, Fig. 1a). Two of 20 (10%) of the CPV titers were in the
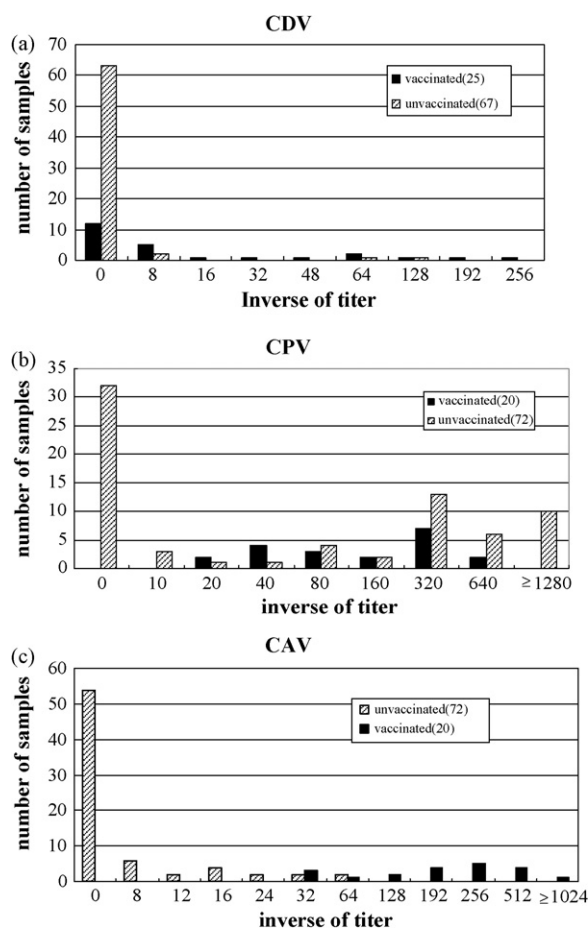

Fig. 1. Distribution of antibody titers by sample number in vaccinated and unvaccinated giant pandas against the following: (a) CDV, (b) CPV, and (c) CAV.

suspect category, 18 of 20 (90%) were positive. Titers in the positive category ranged from 1:40 to 1:640 (Table 3, Fig. 1b). All 20 pandas vaccinated with CAV-1 showed positive (>1:16, 20/20), and the CAV-1 titer of one vaccinated animal was extremely high (≥1:1024), suggesting exposure to a field strain of the virus (Table 3, Fig. 1c).

3.2. Antibody titers in unvaccinated giant pandas

Titers against CDV in unvaccinated giant pandas at WL ranged from negative at 1:8 (63/67) to suspect (1:8 to 1:16, 2/67) to positive (1:16 to 1:128, 2/67; Table 3, Fig. 1a). 94% unvaccinated pandas sampled showed no positive antibody titer against CDV.

Nearly half of CPV titers at WL were positive, with titers up to 1:10,240 (Table 3, Fig. 1b). Dadi was transferred from WL to BJZ in 2004, the CPV titer of this animal rose from 1:320 in April 2004 to 1:1280 in March 2005. The titer of CPV increased fourfold, suggesting that this individual had been exposed to CPV since his arrival at the BJZ. CPV titers in this study did not vary with age and sex ($p = 0.140$ and 0.063, respectively). During the sample period, antibodies

Table 3
Number and percent (in parentheses) of giant panda serum samples with negative, positive or 'suspect' (intermediate) antibody titers against CDV, CPV, CAV, CCV, CPIV in each study location.

		Location		
		WL	CPB[a]	BJZ[b]
Total samples (92)	67	20	5	
CDV	Negative (<1:8)	63 (94)	11 (55)	1 (20)
	Suspect (1:8–1:16)	2 (3)	2 (10)	4 (80)
	Positive (>1:16)	2 (3)	7 (35)	0
CPV	Negative (<1:10)	31 (48)	0	0
	Suspect (1:10–1:20)	4 (4)	2 (10)	1 (20)
	Positive (>1:20)	32 (48)	18 (90)	4 (80)
CAV	Negative (<1:8)	51 (76)	0	3 (60)
	Suspect (1:8–1:16)	10 (15)	0	2 (40)
	Positive (>1:16)	6 (9)	20 (100)	0
CCV	Negative (<1:8)	67 (100)	20 (100)	5 (100)
	Suspect (1:8–1:16)	0	0	0
	Positive (>1:16)	0	0	0
CPIV	Negative (<1:2)	92 (100)	92 (100)	92 (100)
	Positive (>1:2)	0	0	0

[a] Vaccinated with modified live CDV, CCV, CPV-2, CPIV, CAV-1, and rabies virus.
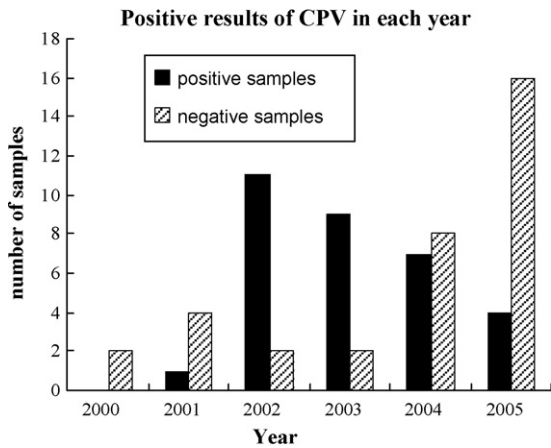[b] Vaccinated with killed CDV.

Fig. 2. Positive and negative results of CPV of unvaccinated giant pandas at WL from 2000 to 2005 (titer range for positive is >1:40).
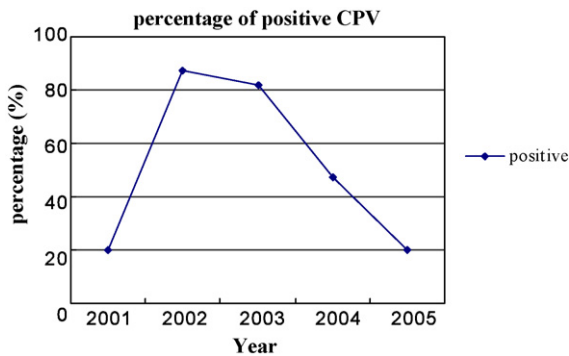

Fig. 3. Percentage of positive CPV of unvaccinated giant pandas at WL from 2001 to 2005 (titer range for positive is >1:40).

against CPV infection increased during 2001 and 2002, then decreased after 2002 (Figs. 2 and 3).

More than half of unvaccinated pandas had no CAV-1 titer (1:8, 54/72), 12 fell into the "suspect" category, and six were positive, with the highest titer 1:64 (Table 3, Fig. 1c).

3.3. Comparison between vaccinated and unvaccinated giant panda groups

Antibody titers against CDV ($p = 0.001$) and CAV-1 ($p = 0.000$) were higher in vaccinated groups than in unvaccinated groups. There was no significant difference for antibody titers against CPV ($p = 0.369$) between vaccinated and unvaccinated groups.

Antibody titers against CCV and CPIV were negative in both unvaccinated and vaccinated groups.

4. Discussion

This study examined the exposure and prevalence of selected canine viruses in vaccinated and unvaccinated giant pandas, and compared antibody titers between the two groups. The results indicate that giant pandas may respond to exposure to field strains of CDV, CPV and CAV-1, and that the vaccines used at the study sites against these pathogens may be of questionable value.

It is not known at what level antibody titers to these pathogens are significant in giant pandas, nor at what level they are protective. A conservative approach to interpretation of the data is to consider low positive titers as "suspect". A low positive titer may suggest nonspecific inhibition in the assay, a waning titer from exposure to the virus (or vaccine), an early stage in seroconversion, or cross-reactivity to a related virus.

CDV is a highly contagious morbillivirus that causes multisystemic disease in a variety of domestic and wild carnivores (Deem et al., 2000). It is believed that the giant panda is susceptible to CDV, and that mortality may be high in some zoos in China (Li et al., 1999; Hu et al., 1997). In this study, 94% of unvaccinated giant pandas had no antibodies against CDV. One explanation may be that the survival rate of infected animals is low and the exposed animals do not exist in the sampled population. CDV is one of the important elements causing diarrhea in giant pandas. Neonates and cubs are prone to diarrhae, which is common in captive giant pandas and is associated with high mortality (Loeffler et al., 2006). Another possible explanation might be that the field virus does not elicit a strong immune response even though it kills the infected animal. The third possibility is that they simply are not exposed to the virus very much.

The prevalence of antibody titers against CPV was much higher than those against CDV in unvaccinated pandas in this study. Nearly half of the unvaccinated animals demonstrated titers against CPV. This is consistent with the findings of Mainka et al. (1994) who detected antibodies against CPV in 60% of giant pandas. Interestingly, the prevalence of positive titers against CPV was similar to a study of captive red pandas in China (Qin et al., 2007b).

Infectious hepatitis caused by CAV-1 has been found in canids, bears and skunks (Williams and Barker, 2001). Only six of the unvaccinated pandas demonstrated titers against CAV-1, with the highest titer 1:64. This suggested that pandas in this study were exposed to CAV-1. He et al. (2004) also detected antibody titers against CAV-1 in 61 unvaccinated giant pandas, and the positive rate was as high as 39.3% (titer ranged from 1:4 to 1:1024).

In this study, vaccine titers against CDV, CPV and CAV-1 varied from negative to highly positive. Titers against CDV ranged from negative (12/25) to suspect (6/25) to positive (7/25), and Dadi did not produce high positive antibody titer (1:8) after vaccination. This suggests that the vaccines used for these animals do not elicit consistent antibody titers. Wang et al. (2008) surveyed the antibody of the canine distemper attenuated live vaccines on 33 giant pandas, and found that the attenuated vaccine was inadequate to stimulate giant pandas to produce high level of antibody against CDV. Based on these observations, and on the widely understood risk of using vaccines in untested exotic species, we suggest that the modified-live and killed canine vaccines are not appropriate for use in giant pandas.

Vaccinated giant pandas carried a high prevalence of positive CPV titers. This is consistent with the findings of Loeffler et al. (2007), who demonstrated that 91% of giant pandas vaccinated with canine vaccine had positive CPV titers. Both studies show high degree of variation in the CPV titers in vaccinated giant pandas. Clearly, the magnitude and distribution of the values in the CPV vaccinated giant pandas in this study did not meet the expectations with an effective vaccine.

The CAV-1 titers of vaccinated pandas were highly variable among individuals, although all CAV-1 titers fell into positive range (>1:16). Loeffler et al. (2007) detected no antibody against CAV-1 in vaccinated individuals, which is different from this result. It is not clear whether the positive titers in the vaccinated group are induced by vaccine or natural exposure. The CAV-1 titer of one vaccinated individual was extremely high, suggesting exposure to a field strain of the virus. This panda was not reported to have shown any signs of illness recently.

A similar variability in antibody titers after vaccination was found in another study of captive red pandas and giant pandas at CPB (Qin et al., 2007a; Loeffler et al., 2007). Both giant and red pandas at CPB were vaccinated with the same attenuated vaccine for dogs. There were no data about the effect and safety of the vaccine in wildlife. As well, the vaccine produces highly variable titers in the two species. The high variability of the vaccine response among individuals suggests that the quality of the vaccine may be inconsistent. Alternative explanations to the viable vaccine titers include inconsistent delivery of the vaccine or variability in response of the giant panda individual to antigens. In China, few trials are conducted to evaluate the effect and safety of vaccine in wildlife; most of experiences were from dogs. In view of the potential risk of attenuated live vaccine to giant panda, it is necessary to assay the effectiveness and safety of these vaccines prior to use.

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.

CCV causes enteritis in canids. Mainka et al. (1994) and Qiao et al. (2004) detected antibody of CCV in giant pandas, and Hu et al. (2004) isolated a CCV strain from one giant panda. However, we did not detected CCV and CPIV antibody in unvaccinated or vaccinated groups in this study. This suggested that CCV and CPIV infections were not prevalent in the study populations or the assays were not sensitive with the panda sera. Another explanation was that the virus strains or their preparation for the vaccine are not appropriate for the immunization of pandas.

In conclusion, our results suggest that exposure of captive giant pandas in some facilities to CDV, CPV and CAV may be of concern, and that the vaccines presently used may be of dubious efficacy and safety. Results of this study re-emphasize the need for research regarding the prevalence, risk, and significance of carnivore infectious diseases in captive giant pandas in China. Furthermore, they emphasize the need for developing new vaccines to protect giant pandas from infectious diseases.

Acknowledgements

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