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Prevalence of Seropositivity to Pathogens in Small Carnivores in Adjacent Areas of Lazovskii Reserve

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Abstract—The prevalence of infectious diseases in wild and feral carnivores is poorly known in Primorsky Krai, where rare species such as the Amur tiger and the Far East leopard roam. In this study we evaluated the prevalence of seropositivity in feral (cats, dogs) and wild (raccoon dog, badger, American mink, Far Eastern wild cat) carnivores to various pathogens: distemper virus, parvovirus, feline immunodeficiency and feline leukemia virus, feline coronavirus, *Toxoplasma gondii, Mycoplasma* sp., and *Chlamydia* sp. It was shown that seropositivity occurred significantly more frequent in feral animals than in wild ones. The highest percentage of seropositive animals was observed in feral dogs. It is these diseases that can be dangerous for the Amur tiger and the Far East leopard, thus affecting their populations.

Keywords: infectious diseases, seropositivity, predator, Amur tiger, Far East leopard
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INTRODUCTION

Infectious diseases pose a serious threat to wild animals, causing depopulation of species (Alexander, Appel, 1994; Roelke-Parker et al., 1996; Murray et al., 1999; Deem et al., 2001). An infection-related population decline has been documented in the lion (*Panthera leo*) and the Ethiopian wolf (*Canis simensis*). The source of infection was feral animals, namely, dogs (Sillero-Zubiri et al., 1996; Cleaveland et al., 2000). Wild canine and feline species are susceptible to diseases of feral cats and dogs (Sillero-Zubiri et al., 1996; Deem et al., 2002). However, little is known about the presence of infectious diseases in wild predatory mammals, in particular, in Primorskii Krai which is the habitat for rare species such as the Siberian tiger (*Panthera tigris altaica*), the Far Eastern leopard (*P. pardus orientalis*), and the Far Eastern Forest Cat (*Prionailurus bengalensis euptilura*). Such studies are very important for areas of likely introduction of the Far Eastern leopard, i.e., the Ussuriiskii and Lazovskii State Nature Reserves, Far East Branch, Russian Academy of Sciences. It is these reserves where we initiated such studies (Esaulova et al., 2009, 2010; Davydova et al., 2010; Naydenko et al., 2011).

The most important diseases of the Amur tiger and the Far Eastern leopard are feline panleukopenia, feline coronaviral enteritis, feline viral immunodeficiency, leukemia, toxoplasmosis, mycoplasmosis, and clamidiosis. The viruses causing feline panleukopenia and canine and mustelid parvoviral enteritis behave similarly in regard to pathogenesis, targeting fast-dividing host cells, which results in diarrhea, suppression of bone marrow activity, and a sharp decline in immunity (Parrish, 1995). Feline coronaviral enteritis is prevalent in 80% of the feral cat population and induces infectious peritonitis. This frequently ends up with death of animals (Pedersen, 1987). Distemper considerably diminished the lion population in 1994 in the Serengeti, killing about 1000 animals (Roelke-Parker et al., 1996). The feline viral immunodeficiency virus strongly related to HIV was detected in a feral cat and other wild feline species (Brown et al., 1994). It induces severe immunodeficiency in feral cats by decreasing T-cell numbers (Torten et al., 1991). The feline leukemia virus has worldwide prevalence and causes anemia, immunodeficiency, and various lymphoid tumors (Hoover et al., 1975; Hardy et al., 1976). Toxoplasmosis caused by the protozoa *Toxoplasma gondii* is a cause of abortions, nonviable offspring, central nervous system disorders, and impairment of the lymphatic and endocrine systems in adult cats, dogs, and other animals (Meli et al., 2009). Mycoplasmosis is primarily associated with conjunctivitis, pneumonia, polyarthritis, gastrointestinal tract diseases, spontaneous miscarriages, and infertility (Meli et al., 2009). Clamidiosis is an acute or chronic disease of cats, dogs, and other animals and is characterized by body temperature elevation, conjunctivitis, coryza, pneumonia, and urogenital disorders. The infection occasionally becomes delayed and shows a chronic course. During infection kittens show acute clinical signs, which tends to end up with death (Meli et al., 2009).

The main objective of this work was to carry out a serological study aimed at determining the prevalence of pathogens in populations of wild and feral predatory mammals that inhabit the Lazovskii State Nature Reserve and adjacent territories.

MATERIALS AND METHODS

Sampling was done across the territory of the Lazovskii State Nature Reserve and adjacent territories from March 2008 to September 2009. The reserve is located in the territory of the similarly named district in the southeastern part of Primorskii krai on the slopes of the Sikhote-Alin mountain range that overlook the Sea of Japan. The total reserve area is 1210 km^2.

The reserve is the habitat for the fox (*Vulpes vulpes*), raccoon dog (*Nyctereutes procyonoides*), Amur badger (*Meles leucurus*), sable (*Martes zibellina*), yellow-throated marten (*Lamprogale flavigula*), yellow weasel (*Mustela sibirica*), the least weasel (*M. nivalis*), common weasel (*M. erminea*), otter (*Lutra lutra*), American mink (*Neovison vision*), Himalayan black bear (*Ursus thibetanus*), Amur tiger, lynx (*Lynx lynx*), and the Far Eastern wild cat. There are 9 major settlements near the reserve. There is a buffer zone occupied by game husbandries with an area of 150 km^2 between settlements and the reserve. Rearing dogs without a leash and cats outdoor as well as illegal dog hunting make it possible for feral animals to come into contact with wild ones. Amur tigers are distributed throughout the reserve and in adjacent areas, regularly coming in close proximity with settlements or even invading them.

Blood was sampled from stray and feral nonvaccinated cats and dogs. Sampling sites are mapped in Fig. 1. To rule out false-positive serological results due to maternal antibodies, only animals 6 months of age or older were bled. To avoid errors due to prior vaccination, owners of cats and dogs were polled before bleeding. Dogs were bled without immobilization using manual fixation. When bled cats were immobilized, injecting intramuscularly tiletamine/zolesepam 10 μg/kg of body weight (Zoletil, Virbak, France).

Wild animals were captured in live animal traps from welded mesh (Havahart Traps, Woodstream Corp., United States) and home-made cages. Fresh fish and chicken were used as bait. Traps were placed in 6 locations (Fig. 1) along roads and pathways every 250 m in the vicinity of reserve cordons. Traps were monitored daily.

Trapped animals were immobilized by injecting Zoletil. Raccoon dogs and badgers were injected with 5 μg/kg of this medicine, Far Eastern wild cats were injected with 10 μg/kg, and American minks were injected with 20 μg/kg. Animals were weighed, photographed, labeled using microchips, clinically evaluated, and bled. In addition, wool and fecal samples were collected, including ectoparasites. After recovery the animal was released at its capture site.

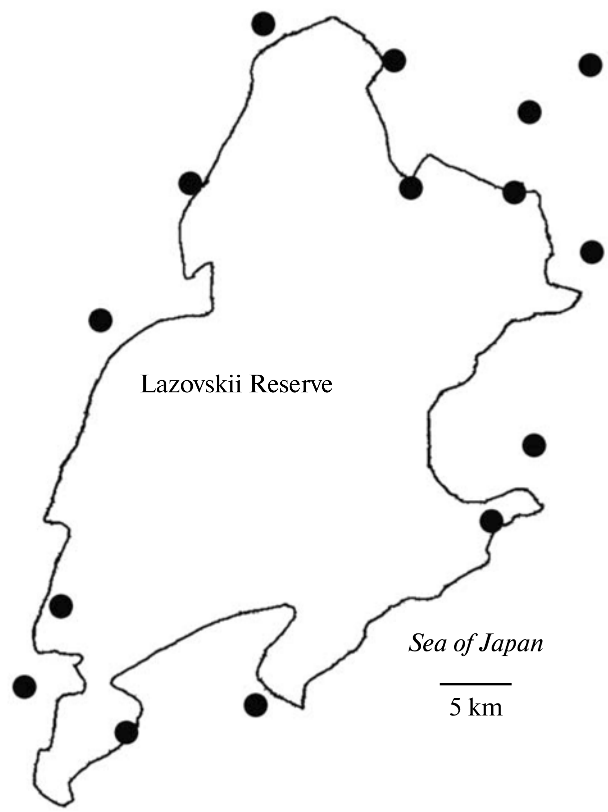

Fig. 1. Capture sites. The line designates the boundaries of Lazovskii Reserve.

All collected blood samples were stored for 2 h and centrifuged at 3000 rpm for 10 min, followed by serum harvest and storage in liquid nitrogen.

Sera in liquid nitrogen were transported to the laboratory. All samples were tested by the Russian Chernogolovka Center for Scientific Research, Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences. All samples were evaluated for seropositivity to parvovirus, distemper virus, chlamydia, *Mycoplasma*, and toxoplasma. All feline sera were tested for antibodies to feline leukemia virus, feline immunodeficiency virus, and feline enteric coronavirus.

Seropositivity to parvoviruses (canine parvovirus, parvovirus of mustelids, and feline panleukopenia) and distemper virus was evaluated by qualitative and quantitative enzyme-linked immunosorbent assay kits (ELISA), produced by Hema-Medika (Russia, Moscow) following the manufacturer's instructions. The two parvoviruses (canine parvovirus, parvovirus of mustelids, and feline panleukopenia) are strongly related, and host antibodies cross-react with both viruses with high affinity. Seropositivity to toxoplasma, Chlamydia, and *Mycoplasma* was also assessed by ELISA commercial kits of the same company without quantitative anal-

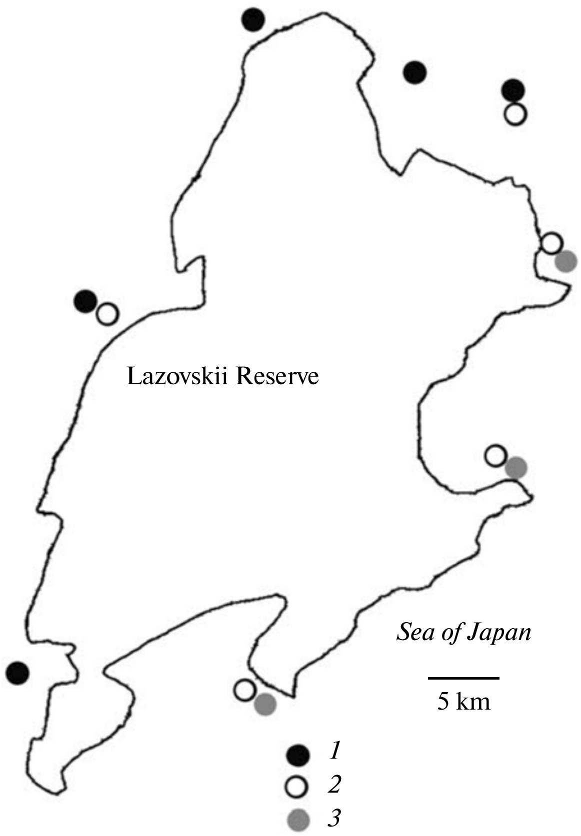

Fig. 2. Sites where animals seropositive for coronaviral enteritis (*1*), distemper (*2*), parvoviral infection/feline panleukopenia (*3*) were found. The line designates the boundaries of Lazovskii Reserve.

ysis (through identification of cut off values). The presence of feline leukemia virus and seropositivity to feline enteric coronavirus and feline immunodeficiency virus were evaluated by BVT rapid immunochromatography kits (France).

Note that seropositivity (occurrence of antibodies) does not imply an infection, but shows prior exposure of an animal to a specific antigen. In addition, animals may also seroconvert to pathogens that are not infectious for a certain species (herein it is the case for distemper virus in feral cats and probably in the Far Eastern forest cat).

Statistics was found by comparing rates. A total of 21 serum samples from feral and stray dogs, 34 serum samples from feral and stray cats, 2 serum samples from Far Eastern forest cats, 5 serum samples from Amur badgers, 3 serum samples from American minks, and 5 serum samples from raccoon dogs (in total 15 serum samples from wild predatory mammals). Far Eastern forest cats and feral cats could be affected by all the pathogens that are infectious to a tiger (except distemper virus). Amur badgers and raccoons dogs are important prey for the Amur tiger.

RESULTS

Serology results are given in the table.

Seropositivity to distemper virus was found in 16% of the animals. A suspect result was identified in 4 feral dogs and 1 raccoon dog. Seropositivity occurred only in feral dogs (52%). According to the statistics, this difference was statistically significant at the rate of seropositivity in wild canine and mustelid species ($p < 0.01$). Seropositive animals were found west, south, and east of Lazovskii district (Fig. 2).

Seropositivity to parvovirus (the etiological agent of enteritis in canine and mustelid species and feline panleukopenia) occurred in 9% of the animals. The same percentage of animals (9%) was identified as suspect (4 feral dogs and 2 raccoon dogs). Seropositivity was only found in feral dogs. The frequency of seropositivity in feral dogs showed statistical significance over feral cats ($p < 0.01$). No significant difference in the frequency of seropositivity was found between wild and feral predatory mammals, feral dogs, and wild canine and mustelid species. Seropositive animals were found south and east of Lazovskii Reserve (Fig. 2).

Nineteen percent of the sera collected from feline animals tested positive for antibodies to coronavirus ($n = 36$). Only stray and wild cats were seropositive; two Far Eastern forest cats trapped were seronegative. Seropositive animals were found north, west, and southwest of Lazovskii Reserve (Fig. 2).

No feline leukemia virus antigen was detected in sera from feral cats. Only one feral cat was found seropositive to feline immunodeficiency virus (3%) (Fig. 3).

Toxoplasma seropositivity was detected in 16% of the animals tested. Of seropositive animals, 75% were feral dogs (38% of dogs were seropositive); however, *Toxoplasma gondii* antibodies were also identified in one Far Eastern forest cat and one American mink. No significant difference in frequency of seropositivity was generally seen in wild and feral carnivores, although it occurred statistically more frequently in feral dogs over wild canine and mustelid species ($p < 0.05$) and feral cats ($p < 0.001$). An overwhelming majority of seropositive animals were found east of Lazovskii Reserve; one case was observed in the south of an area close to Petrovskaya Pad (Fig. 3).

Chlamydia seropositive animals were 6%. *Chlamydia* antibodies were found only in feral and stray dogs (19%). No statistical significance in the frequency of seropositivity was observed among wild and feral carnivores, feral dogs, and wild canine and mustelid species, although *Chlamydia* seropositivity was numerically more frequent in feral dogs over feral cats ($p < 0.05$). Seropositive animals were found east of Lazovskii Reserve (Fig. 4).

Antibodies to *Micoplasma* were observed in 36% of the animals tested. Of feral and stray dogs, 90% were seropositive to *Mycoplasma*. All raccoon dogs and 1 stray cat also showed seropositivity to *Mycoplasma*

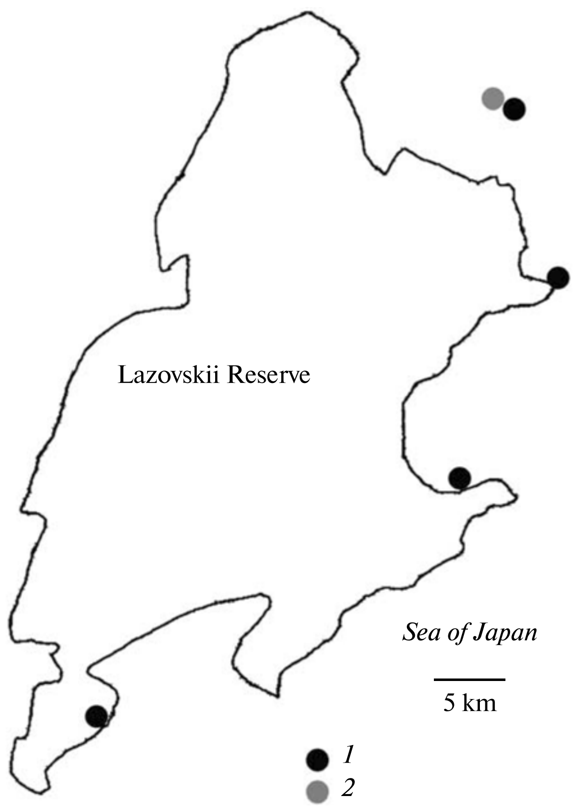

Fig. 3. Sites where animals seropositive for toxoplasmosis (*1*) and feline immunodeficiency (*2*) were found. The line designates the boundaries of Lazovskii Reserve.

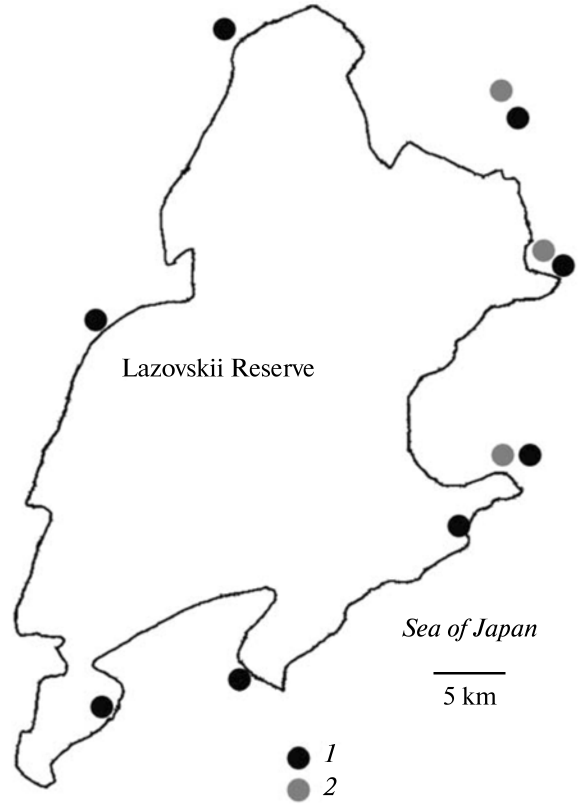

Fig. 4. Sites where animals seropositive for mycoplasmosis (*1*) and chlamidiosis (*2*). The line designates the boundaries of Lazovskii Reserve.

infection (Fig. 4). No statistical significance in the frequency of seropositivity was observed between wild and feral carnivores, although the frequency of seropositivity in feral dogs statistically differed from wild canine and mustelid species ($p < 0.01$) and feral cats ($p < 0.001$).

DISCUSSION

Serology results showed that feral dogs had the highest prevalence of seropositivity. Dog owners do not tend to vaccinate their dogs against distemper and parvoviral enteritis, which, by itself, poses the threat of an outbreak of these diseases. Fifty percent of seropositivity to distemper virus and about 25% of seropositivity to parvovirus in feral dogs show that dogs have prior exposure to the viruses; they carry the viruses, and that the viruses are widely distributed across these populations.

Feline coronavirus was also documented across this territory. Despite the fact that the original virus is avirulent for cats, the threat is posed by a mutant variant of the virus that causes peritonitis and death (Herrewegh et al., 1997). This virulent mutant emerges in about 10% of infected cats (Vennema et al., 1998).

Considering that the leukemia virus is highly transmissible and no leukemia virus antigen was detected in feline sera, we suggest that this virus has no distribution across the territory or it is confined to cat populations not included in this study.

The occurrence of antibodies to *Mycoplasma* agents is ambiguous to interpret. In terms of tiger infections, only one *Mycoplasma* species, *Mycoplasma felis*, is of importance, capable of affecting feline species, whereas in this study we detected generic antibodies to all species from the *Mycoplasma* genus, which includes many species. Overall, a positive result is difficult to interpret unambiguously; however, a negative result is much unambiguous in this case: 64.3% of the animals tested had no prior exposure to mycoplasmas, including pathogenic *M. felis*. Only canine animals were seropositive (only one stray cat showed mycoplasma seropositivity); therefore, it is suggested that *M. felis* is not a threat for feline species inhabiting Lazovskii Reserve and adjacent areas.

Similarly, seropositivity to *Chlamydia* and *Chlomydophila* is ambiguous. Of greatest importance for us was only *Chamydophila felis*. The absence of chlamydia antibodies in feline animals shows that the animals tested may have had no exposure to pathogenic *Chlamydia* and *Chlomydophila* species.

Among wild predatory mammals of Lazovskii Reserve, seropositivity was observed to two pathogens:

Serology testing of blood samples taken from animals inhabiting the Lazovskii State Nature Reserve and adjacent territories

Species	Distemper	Parvoviral enteritis/ feline panleukopenia	Coronoviral enteritis/feline infectious peritonitis	Feline leukemia virus	Feline immunodeficiency virus	Toxoplasmosis	Chlamidiosis	Mycoplasmosis
The Far Eastern forest cat	0/2	0/2	0/2	0/2	0/2	1/2	0/2	0/2
American minks	0/3	0/3	–	–	–	1/3	0/3	0/3
Badgers	0/5	0/5	–	–	–	0/5	0/5	0/5
Raccoon dogs	0/5	0/5	–	–	–	0/5	0/5	5/5
Wild animals (% seropositivity)	**0**	**0**	**–**	**–**	**–**	**13**	**0**	**33**
Feral and stray cats	0/34	0/34	7/34	0/34	1/34	1/34	0/34	1/34
Feral and stray dogs	11/21	5/21	–	–	–	9/21	4/21	19/21
Feral animals (% seropositivity)	**20**	**9**	**21**	**0**	**3**	**18**	**7**	**36**
Total (% seropositivity)	16	7	19	0	3	17	6	36

Note: The number of seropositive samples/the number of tested samples from a certain animal species.

toxoplasma (13%, American mink and Far Eastern forest cat) and mycopalsma (33%, raccoon dogs). No seropositivity to other pathogens was found. Moreover, the absence of antibodies cannot ensure maintenance of a population. For a small population, a disease incursion into one or two animals threatens most of the population like the leukemia virus outbreak in the Iberian lynx (*Lynx pardina*) in the Doñana National Park (Meli et al., 2009).

In feral animals we found seropositivity to 7 out of 8 pathogens tested for. Besides toxoplasma and mycoplasma, seropositivity was observed to distemper virus, parvoviruses (including feline panleukopenia), immunodeficiency virus, enteric coronavirus, and chlamydia. The percentage of feral animals seropositive to a number of pathogens is similar to that of wild animals. Statistical differences were only observed for distemper virus in wild and feral animals (feral animals had a statistically significant frequency). Importantly, feral cats are not affected by distemper, and no seropositive result was identified. Distemper seropositive dogs were over 50%. Dog hunting (including poaching) and rearing dogs without a leash could increase a probability of contacts of Amur tigers with virus carries (Seredkin et al., 2010). *Panthera* species are sensitive to distemper virus: there is evidence that it is capable of causing neural system disorders in Amur tigers and ultimately death of tigers (Quigley et al., 2010). In addition, the occurrence of distemper virus in tigers is quite high (Naydenko et al., 2011).

In summary, infections pose a potential threat to Amur tigers inhabiting Lazovskii Reserve and to the Far Eastern leopard to be introduced into the reserve territory. One of the ways to resolve the problem is to mass vaccinate all feral dogs as part of programs aimed at studying and conserving the Amur tiger and the Far Eastern leopard implemented by the Russian Academy of Sciences, the Zoological Society of London, and Wildlife Conservation Society.

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