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ARTICLE

Patterns of seroprevalence of feline viruses among domestic cats (*Felis catus*) and Pallas' cats (*Otocolobus manul*) in Daursky Reserve, Russia

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Abstract: Few data are available on the prevalence of feline viruses in the wild and little is known about natural sources of infections. The aim of this study was to estimate patterns of seroprevalence to feline viruses (feline immunodeficiency virus (FIV), feline calicivirus (FCV), feline panleukopenia virus (FPV), feline herpesvirus (FHV), and feline leukemia virus (FeLV)) in two cat species, domestic cats (*Felis catus* L., 1758) (n = 61) and Pallas' cats (*Otocolobus manul* (Pallas, 1776)) (n = 24), living in the same area, in Daursky Reserve, Russia. Our results indicate that four of five viruses are circulating in the study area, with the exception of FHV. The pattern of FCV and FPV prevalence differed from FIV and FeLV. FCV and FPV seroprevalence did not depend on the sex and predominated in the group of cats living in the village (76% and 55%, respectively). No Pallas' cats were seropositive to these viruses. The prevalence of FIV and FeLV were similar in areas with different cat densities (at the stations (16% for both viruses) and in the village (16% for both viruses)). The patterns of seroprevalence between species testify to the low rate of interspecific contacts. In Pallas' cats, we found the presence of antibodies to FIV to be 5% and antigens of FeLV to be 5%, pathogens for which transmission demand close direct contacts between animals (mainly aggressive and (or) sexual contact), which is typical in the breeding season. Arid climate may also reduce patterns of viral prevalence in the study area, decreasing the risk of infection for both cat species.

Key words: Pallas' cat, *Otocolobus manul*, domestic cat, *Felis catus*, seroprevalence, feline viruses, natural populations.

Résumé : Peu de données sont disponibles sur la prévalence des virus félins dans la nature et les connaissances sur les sources naturelles d'infection sont très limitées. L'étude avait pour objectif l'estimation des motifs de séroprévalence de virus félins (virus de l'immunodéficience féline (FIV), calicivirus félin (FCV), virus de la panleucopénie féline (FPV), herpès-virus félin (FHV) et virus de la leucose féline (FeLV)) chez deux espèces de chats, le chat domestique (*Felis catus* L., 1758) (n = 61) et le manul (*Otocolobus manul* (Pallas, 1776)) (n = 24), vivant dans la même région, dans la réserve de Daursky (Russie). Nos résultats indiquent que quatre des cinq virus circulent dans la région d'étude, le FHV faisant exception. Les motifs de prévalence du FCV et du FPV étaient différents de ceux du FIV et du FeLV. Les séroprévalences du FCV et du FPV ne dépendaient pas du sexe et étaient prédominantes dans le groupe de chats vivant dans le village (76 % et 55 %, respectivement). Aucun manul n'était séropositif pour l'un ou l'autre de ces virus. Les prévalences du FIV et du FeLV étaient semblables dans des zones caractérisées par différentes densités de chats (aux stations (16 % pour les deux virus) et au village (16 % pour les deux virus)). Les motifs de séroprévalence des deux espèces témoignent de la faible fréquence des contacts interspécifiques. Chez les manuls, nous avons observé une présence d'anticorps pour le FIV de 5 % et des antigènes du FeLV, 5 %, des pathogènes dont la transmission nécessite des contacts directs entre animaux (contacts principalement sexuels ou d'agression), une situation typique durant la saison de reproduction. Un climat aride pourrait également limiter la prévalence virale dans la région à l'étude, réduisant le risque d'infection pour les deux espèces de chats. [Traduit par la Rédaction]

Mots-clés : manul, *Otocolobus manul*, chat domestique, *Felis catus*, séroprévalence, virus félins, populations naturelles.

Introduction

Infectious diseases may affect the distribution and abundance of animals, but the potential role of diseases in wildlife conservation has only recently drawn considerable attention (Scott 1988; Macdonald 1993, 1996). Many species or populations of Felidae are already seriously threatened by different factors (Wildt et al. 1987; Garrote et al. 2013; Seimon et al. 2013). Among them the role of pathogens has remained the least investigated; additionally little is known about natural sources of these infectious agents. Domestic species, such as domestic dogs (*Canis lupus familiaris* L., 1758) and domestic cats (*Felis catus* L., 1758), are considered a potential source of different infections because wild cat species are susceptible to a wide array of highly lethal or debilitating pathogens, many of which are either endemic to or easily transmitted by domestic animals (Roelke-Parker et al. 1996; Daniels et al. 1999; Goncharuk et al. 2012; Bevins et al. 2012). The Pallas' cat (*Otocolobus manul* (Pallas, 1776)) is a small, solitary cat species, with a broad but fragmented distribution, that is both poorly studied and endangered. High susceptibility of Pallas' cats to different common feline infections has been described in captivity, especially to *Toxoplasma gondii* (Nicolle and Manceaux, 1908) (Dubey et al. 1988; Ketz-Riley et al. 2003; Basso et al. 2005). Also, feline immunodeficiency virus (FIV) was isolated in a captive Pallas' cat (Barr et al.

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1997). Young Pallas' cats in North American zoological parks have died from *T. gondii* infections, but these animals also had concurrent infections, such as feline panleukopenia virus (FPV) or feline herpesvirus (FHV) (Ketz-Riley et al. 2003). Among Pallas' cats examined post mortem at Moscow Zoo, there were animals with signs of feline leukemia virus (FeLV), feline calicivirus (FCV), FIV, and FPV (unpublished data from Moscow Zoo). However, little is known about the seroprevalence of pathogens in free-ranging Pallas' cats, especially virus infections, and their natural sources in the wild. In previous studies, FIV antibodies were found in 25% ($n = 28$) of Pallas' cats surveyed in Mongolia (Brown et al. 2010), but antibodies against coronavirus and the presence of FeLV antigen were not found (Brown et al. 2005). By contrast, there was no evidence of FIV antibodies in Pallas' cats ($n = 10$) surveyed in Daursky Reserve, Russia, and the seroprevalence of FeLV was 7.7% ($n = 13$) (Naidenko et al. 2014). At the same location, the mortalities of three Pallas' cats attributed to infections of unknown etiology had also been recorded earlier (probably FPV) (Kirilyuk and Puzanskii 2000). Thus, Pallas' cats are known to be susceptible to most common feline viruses such as FIV, FeLV, FHV, FCV, and FPV, with infections recorded both in captivity and in the wild.

In the wild, free-ranging domestic cats may be an additional source of the common viruses besides conspecifics in Pallas' cats. However, in a previous study in Mongolia, there was no evidence that domestic cats were a potential reservoir of FIV to Pallas' cats, although this may be because the sample size was too small ($n = 15$). Moreover, Mongolians typically do not maintain domestic cats as companion animals, limiting the opportunity for viral transmission between domestic cats and Pallas' cats in the country (Brown et al. 2010). In the buffer zone of Daursky Reserve, the south of Zabaikalskii Kray, Russia, where our study was conducted, both cat species inhabit the same area. In contrast to Mongolia, local people traditionally keep domestic cats that, therefore, inhabit almost every house in villages, as well as at herdsmen stations located in this area. All local domestic cats are free-ranging owned animals, have both indoor and outdoor access, but depend on food and shelter provided by owners. At the same time, population density differed between domestic cats from a village and herdsmen stations, determined by the structure of human housing. Domestic cats from herdsmen stations, supposedly, have larger home ranges than the ones from a village. Density of animals was lower at herdsmen stations; cats could be less socialized and more aggressive to neighbors than their village fellows (Liberg 1980). Based on little data, the Pallas' cat has a typical solitary life style. The size of home ranges fluctuates widely, depending mainly on the sex (female: 1.3–22.0 km^2; male: 5.7–50.3 km^2) and the habitat configuration, rather than on prey resources (Ross 2009). Population density of Pallas' cats is estimated from 0.016 to 1.6 cats/km^2 in different parts of the Russian range (Kirilyuk and Puzanskii 2000). Under such conditions, we expected that the frequency of intraspecific contacts between Pallas' cats to be significantly lower than intraspecific contacts of domestic cats in the same area and maybe even lower than interspecific contacts. We expected that the seroprevalence of the five viruses previously mentioned in Pallas' cats may differ, due to differences in their characteristics, transmission modes, and possibly differences in population densities linked to cat behavior (frequency of social contacts, their type (amicable, sexual, or aggressive), and possibility to contact with infected environment). FCV and FHV are highly contagious pathogens with a widespread distribution in feline populations (with prevalence commonly exceeding 50%) (Gaskell et al. 2007; Radford et al. 2009; Hellard et al. 2011). Both viruses are shed predominantly by ocular, nasal, and oral secretions and transmission is largely by direct contact with an infected cat (Povey and Johnson 1970). These viruses infected unowned cats more frequently than owned cats and the prevalence is higher in large groups of animals housed together than in cats kept in small groups (Radford et al. 2009). Young unowned cats are infected by FHV more frequently than adult ones, whereas the prevalence of FCV is higher among adult animals. Additionally, FCV can persist in the environment for about 1 month, whereas FHV is relatively short-lived outside the host and quickly decay in open air (Gaskell et al. 2007; Radford et al. 2009). Infections with FPV have been described in many different cat species. The virus results in high mortality in unvaccinated populations of domestic cats (Steinel et al. 2001). FPV is unique among the viruses in our study, as it is transmitted predominately through indirect contacts with the infected environment because the virus is excreted mainly in feces, but also in urine, saliva, and vomit. Moreover, FPV has high resistance in the environment and stays infectious for more than 12 months (Clay et al. 2006; Hellard et al. 2011). Owned cats, which tend to live more closely together, were more frequently infected with FPV than unowned cats that survive on hunted prey and tend to have larger and less populated home ranges. FeLV and FIV occur worldwide in domestic cats and lead to immunosuppression and opportunistic infections in infected animals (Hofmann-Lehmann et al. 1996). The prevalence of FeLV and FIV is usually lower than for the previous viruses in populations of domestic cats (<20%) (Dorny et al. 2002; Lee et al. 2002; Gleich et al. 2009; Hellard et al. 2011). Both viruses require direct contact between cats for their transmission. FIV is mainly transmitted by bites, through a direct horizontal mode (Sparger 1993), and the spread of the virus depends on the frequency of aggressive and sexual contacts between animals (Fromont et al. 1997). Previous studies showed that FIV is more prevalent in unowned cats, presumably because they are more aggressive and territorial than owned cats, with higher prevalence in males than in females because aggressive dominant males are likely to be bitten during fights (Hellard et al. 2011). FeLV is transmitted via the saliva or blood through direct contact: not only through biting, but also mainly during licking and grooming, or from mother to fetus during pregnancy. Thus, broader range of social relations promotes the spread of FeLV than FIV and FeLV is more prevalent among social active cats kept in the same household (Fromont et al. 1997). Additionally, both viruses cannot persist in the environment for a long time and quickly decay in open air (Hardy et al. 1975; Fromont et al. 1997).

The aim of this study was to estimate patterns of seroprevalence of feline viruses in the Daursky Reserve, Russia. Thus, we estimate the seroprevalence of five viruses (FCV, FHV, FPV, FeLV, FIV) in two cat species, domestic cats and Pallas' cats, that inhabit the same area. We also assessed intraspecific seroprevalence differences depending on sex and animal density in domestic cats, as well as interspecific seroprevalence differences, that may be linked to variation in species ecology, in particular spatial and social organizations. Considering the modes of transmission of the five viruses, we expect that the seroprevalence of FCV, FHV, and FPV will depend mainly on the population density (Steinel et al. 2001; Gaskell et al. 2007; Radford et al. 2009), while FeLV and FIV prevalence may be dependent on the character of social contacts (Fromont et al. 1997). Therefore, we predict that the number of FCV-, FHV-, and FPV-positive animals will be higher among domestic cats in the village than at the stations and lower among Pallas' cats than in domestic cats. We also assume that the ratio of aggressive contacts to other social contacts does not significantly differ between domestic cats in the village and at the stations, as recorded elsewhere among urban and rural cats (Liberg et al. 2000). However, the frequency of any social contacts is higher among domestic cats than Pallas' cats in the study area. Therefore, we predict that there will be interspecific differences in the prevalence of FIV and FeLV, but not intraspecific ones. In addition, we suppose that there are sexual differences in the prevalence of FIV and FeLV, but not in other tested viruses in both species.

Table 1. Demographic information for 61 free-ranging domestic cats (*Felis catus*) and 24 wild Pallas' cats (*Otocolobus manul*) caught in the buffer zone of the Daursky Reserve, Russia.

Cat	Sample year	Age (adult:subadults)	Sex (male:female)	Range	Density (no. of animals/km²)
Domestic cat	2013 (Feb.–Mar.)	28:0	14:14	Herdsman station (Mergen, Borsianka River)	0.25
	2013 (Oct.–Nov.)	33:0	23:10	Kulusutaii (near Mergen)	63
Pallas' cat	2011 (Feb.–Mar.)*	13:4*	10:7*	Mergen*	nd*
	2012 (Mar.)	4:0	0:4	Mergen	nd
	2014 (Feb.–Mar.)	3:0	2:1	Mergen	nd

Note: The village of Kulusutaii is located near the Mergen Peninsula, where herdsman stations are located and where the most tested Pallas' cats were captured. Age is based on the ratio of adults to subadults, where an adult cat is >1 year old and a subadult cat is about 1 year old. nd, not determined in this study.

*Data are from Naidenko et al. (2014).

Materials and methods

Study area

The study was conducted in the buffer zone of Daursky State Natural Biosphere Reserve located south of Zabaikalskii Kray near the Russian–Mongolian border (50.06°N, 115.44°E). The study area covered approximately 425 km² and included the typical habitats of the Pallas' cat, i.e., dry mountain steppes with hills covered with quite short grass (the height is less 30 cm) without upright trees. This area is one of the driest and coldest regions of the Central Asian steppes: annual precipitation here is 150–400 mm and annual temperature fluctuation can exceed 90 °C (maximum summer temperatures can be +49 °C in the shadow and minimum winter temperatures are −45 to −47 °C). Additionally, there are herds of domestic livestock (sheep, horses, cows, camels) and also wild Mongolian gazelles (*Procapra gutturosa* (Pallas, 1777)). The study area included the Mergen Peninsula on Lake Borun-Torey (50.15°N, 115.58°E) (16 km²), surrounding territories without water, and the area around the village of Dauria (49.92°N, 116.85°E). Within this area, the village of Kulusutaii (50.23°N, 115.68°E) and 23 herdsmen stations were explored. Sixty-one domestic cats and 24 Pallas' cats were captured between 2011 and 2014 (Table 1). The area of the village was 1.028 km² and contained 164 buildings, including dwelling houses, sheds, and hangars. Based on the survey (65 cats were counted for the village), we estimated the density of adult cats as 63 individuals per 1 km². We sampled approximately 50% of the population of village cats randomly and 33 cats were sampled. Additionally, 28 animals were sampled at all herdsmen stations. Every station was mapped with GPS (Garmin 62CSX). The mean distance between the nearest herdsmen stations was 4.5 km. Free-ranging domestic cats can cover such distance easily (Naidenko and Hupe 2002) and can move between adjacent stations. We estimated the total area (where we checked 23 herdsmen stations) using the minimum convex polygon (MCP) method. The total area was 112 km². The density of domestic cats in the herdsmen stations (28 animals/112 km²) was evidently lower (0.25 cat/km²) than in the village. All tested domestic cats were adults (>1 year old). All cats (from the village and stations) were neither vaccinated nor neutered. Our observations and the survey of local people showed that there were no absolutely unowned cats in the study area. All cats had shelters, mainly in houses and sometimes in shops, the post office, or school, and were always provided with food and water. The majority of the Pallas' cats were captured at the Mergen Peninsula in 2011 (Naidenko et al. 2014); the rest of the animals were sampled within the entire study area. Only 4 of 24 animals were classified as subadult (about 1 year) based on the condition of teeth and body mass (Table 1), while the rest were classified as adult and could not be aged precisely.

Sampling

Every domestic cat was handled directly by the owner. Blood samples were obtained from the femoral vein (1–2 mL) within 5 min without anaesthesia. Methods of capture and sample collection from Pallas' cats' have been described in detail previously (Naidenko et al. 2014). After sampling, blood was placed into Eppendorf tubes (Scientific Specialties, Inc. (SSI), Lodi, California, USA) for serological analyses. Blood samples for serological analysis were kept cool prior to processing for a maximum of 3 h to minimize haemolysis of red blood cells. After centrifugation (20 min at the rate 6000 rev/min), serum samples were frozen and stored at −18 °C until analysis (about 1–2 months). Serological analysis was conducted at the A.N. Severtsov Institute of Ecology and Evolution, Moscow, Russia.

Serological analysis

Serum samples from domestic cats were tested for antibodies against FPV, FCV, FHV, and FIV and for antigens of FeLV. The serum prevalence of FIV, FPV, FHV, and FeLV (but not FCV) was published in Pallas' cats caught in 2011 (Naidenko et al. 2014). In the current study, antibodies against FPV, FHV, and FIV and antigens for FeLV were measured in newly caught Pallas' cats; additionally, the most serum samples were analyzed for the presence of FCV antibodies (Table 2). The presence of FCV, FHV, and FPV antibodies was detected by commercial enzyme-linked immunosorbent assay (Immunocomb®; Biogal, Galed Labs. Acs Ltd., Kibbutz Galed, Israel) according to the manufacturer's instructions. Animals were considered serum positive with a titre of 1:32 or higher for FCV and serum negative with a titre less than 1:32; animals were considered serum positive with a titre of 1:16 or higher for FHV (serum negative with a titre less than 1:16) and serum positive with a titre of 1:80 or higher for FPV (serum negative with a titre less than 1:80) according to the manufacturer's instruction. These kits were produced to detect antibodies to the pathogens and were previously tested successfully on the captive Pallas' cat from the Volokolamsk (Russia) breeding centre. The immunochromatography tests (snap tests; Bio Veto Tests (BVT) Groupe Virbac, La Seyne sur Mer, France) were used to detect the presence of FeLV group-specific antigen, as well as the presence of FIV antibodies. According to the manufacturer, this test's sensitivity and specificity are both higher than 94% (sensitivity: FeLV = 94.7%, FIV = 96.3%; specificity: FeLV = 99.2%, FIV = 98.9%). The detection of FeLV has been done through the detection of a specific antigen. This approach is used quite often (Gleich et al 2009; Hellard et al. 2011). Thus, we considered the animals to be the positive cats that developed FeLV-related diseases. It leads to some underestimation of seropositive animals because some individuals with detectable proviremia did not show antigen presence. However, the same approach was used for both cat species (i.e., domestic cats and Pallas' cats), thus making the data sets comparable.

Studied factors

We based our estimates of animal density on our own data and literary sources as the main factor that influenced the serum prevalence of tested viruses. Within domestic cats, the animal density

Table 2. Description of the structure of the samples according to the studied parameters and apparent prevalence (proportion of positive individuals among tested ones) for the five viruses.

	FHV	FCV	FPV	FIV	FeLV
Domestic cat (*Felis catus*)					
Sample size (*n*)	61	60	60	58	58
Prevalence (%)	0	61.7	45.0	10.3	10.3
Females (%)	24 (0)	24 (54.2)	24 (54.2)	23 (17.3)	22 (18.2)
Males (%)	37 (0)	36 (66.7)	36 (38.9)	35 (17.4)	36 (18.2)
Herdsman stations (%)	28 (0)	27 (44.4)	27 (33.3)	25 (16.0)	25 (16.0)
Kulusutaii (%)	33 (0)	33 (75.8)	33 (54.5)	33 (6.1)	33 (6.1)
Pallas' cat (*Otocolobus manul*)					
Sample size (n)	20	20	20	19	21
Prevalence (%)	0	0	0	5.3	4.7
Females (%)	11 (0)	12 (0)	12 (0)	11 (9.1)	11 (9.1)
Males (%)	9 (0)	8 (0)	8 (0)	8 (0)	10 (0)

Note: FHV, feline herpesvirus; FCV, feline calicivirus; FPV, feline panleukopenia virus; FIV, feline immunodeficiency virus; FeLV, feline leukemia virus.

had two modalities (high density of animals in the village and low density of animals at the stations) as previously defined. We also conducted interspecies comparison of the serum prevalence, suggesting that the density of Pallas' cats is lower than domestic cats within the study area. Additionally, we assessed the influence of sex (male and female) on the serum prevalence in domestic cats because the sex ratio was shifted toward males in the village, where owners prefer keeping males.

Statistical analysis

FHV risk factors could not be investigated because there were no positive animals. Also, FeLV and FIV risk factors could not be investigated for Pallas' cats because the number of positive individuals was too low. In domestic cats, we analyzed effects of sex and population density and their interaction on FCV, FPV, FeLV, and FIV risk factors (positive or negative) using generalized linear models (McCullagh, Nelder 1989) for binomial distribution with logit-link function (separate model for each virus). Cochran's *Q* test for related samples was performed to compare the level of seroprevalence (positive or negative) for different viruses in the sample of domestic cats. To compare the percentage of serum positive Pallas' cats and domestic cats, the two-tailed Fisher's exact test was used. The 95% level of confidence was calculated and $p < 0.05$ was considered to be statistically significant. Statistical analyses were conducted using Microsoft® Excel® (Microsoft Corporation, Redmond, Washington, USA) and Statistica version 8.0 (StatSoft, Inc., Tulsa, Oklahoma, USA).

Results

Among all tested domestic cats and Pallas' cats, FHV was not detected. FCV and FPV were found only in domestic cats, whereas FIV and FeLV were detected in both tested cat species.

Intraspecific differences in the seroprevalence

We found that the level of seroprevalences of different viruses was significantly different (Cochran's *Q* test: $Q_{|3|} = 54.98$, $p < 0.001$) in domestic cats. The frequency of animals being positive for FCV (62%; $n = 60$) and FPV (45%; $n = 60$) was higher than for FIV (10%; $n = 58$) and FeLV (10%; $n = 58$). The effect of animal density was significant, whereas the effect of sex was not significant, for FCV and FPV in the generalized linear model (Table 3): domestic cats in the village were positive for these viruses more frequently (76% and 54%, respectively) than domestic cats at the herdsman stations (44% and 33%, respectively) (Table 2). For FeLV and FIV, both effects of density and sex were not found to be significant in domestic cats (Table 3). However, the tendency was opposite when compared with FCV and FPV: the domestic cats at the herdsman stations were infected by FeLV (16%) and FIV (16%) ($n = 28$) slightly more frequently than domestic cats from the village ($n = 33$) (the seroprevalence of both viruses was 6%).

Interspecific differences in the seroprevalence

The seroprevalence of FIV ($n = 12$) and FeLV ($n = 14$) were analysed in the Pallas' cats caught in 2011. We found only one female positive for FeLV (7%) (Naidenko et al. 2014). Seven newly caught Pallas' cats were also tested for FIV and FeLV. All of these Pallas' cats were negative for the FeLV antigen. Therefore, the serum prevalence of FeLV was 5% (total $n = 21$). However, antibodies against FIV were detected in one female living near the Russian–Mongolian border (5%; $n = 19$) (Table 2). We found significant differences between the seroprevalences of FCV and FPV in Pallas' cats ($n = 20$) and domestic cats ($n = 56$) (Fisher's exact test: $p < 0.001$), but not between FIV and FeLV ($n_1 = 19$–21; $n_2 = 53$; $p > 0.05$) (Table 2).

Discussion

There is little information on the prevalence of feline pathogens in natural cat populations. This is the first time that patterns of seroprevalence of five viruses were assessed in two cat species living in the same area, the domestic cat and the Pallas' cat. In addition, this is the first study to perform comparative analyses on the prevalence of feline viruses in two cat species, which have differences in ecology, spatial and social organizations, and also using food resources and interactions with human beings.

We found the presence of antibodies against four of five tested viruses (FCV, FPV, FeLV, and FIV, but not FHV) in two cat species in the study area. Surprisingly, FHV-positive animals were not found. This virus is quite common, at least in domestic cats (Gaskell et al. 2007; Hellard et al. 2011). We suppose that the lack of FHV prevalence is determined by the instability of the virus in the environment, as well as the low number of juvenile animals of both species (only 4 subadults among 24 Pallas' cats in our samples sets) that are more susceptible to the virus (Gaskell et al. 2007).

Intraspecific differences in the seroprevalence

Consistent with other studies (Radford et al. 2009; Hellard et al. 2011), the seroprevalence of FCV and FPV depended on population density and was higher in domestic cats in the village than the ones at the herdsmen stations. In spite of the differences in transmission modes of these viruses, the seroprevalence is usually higher in large groups of animals housed together. In such conditions, there is a greater possibility of contact between conspecific excreta (the main transmission rout of FPV) and conspecifics (the main transmission route of FCV) than in household cats kept in small groups (Radford et al. 2009; Steinel et al. 2001). In the study area, distances to the nearest neighbors were greater in domestic cats living at the herdsmen stations (3–6 km). Therefore, frequency of direct contacts between animals is likely to be lower than between domestic cats in the village. It might be one of the main causes of lower FCV prevalence in domestic cats at the herdsmen stations than those cats residing in the village. Moreover, at the herdsmen stations, there is less possibility of contact with feces, and feces-contaminated objects within one station, and even less so between neighboring stations, which may explain why infection with FPV is lower in domestic cats at the stations compared with cats in the village.

The total FIV and FeLV seroprevalences (10.3% for both) were lower than for all other viruses in domestic cats in the study area. This was consistent with the findings of previous studies on feral and rural cats (Fromont et al. 1997, 1998; Pontier et al. 1998; Hellard et al. 2011). Consistent with our prediction, the seroprevalence of FIV and FeLV was statistically similar in cats living in territories with different population density. However, the tendency was opposite to the patterns recorded for FCV and FPV, with a higher number of positive animals at the herdsmen stations than in the village. It is possible that the domestic cats at the

Table 3. Effects of population density (high vs. low) and sex (male vs. female) on the prevalence of antibodies to the four viruses in serum of domestic cats (*Felis catus*) in the buffer area of the Daursky Reserve, Russia.

		Effects of density (high vs. low)				Effect of sex (male vs. female)			
Virus	Sample size (*n*)	*B*	SE	χ^2	*p*	*B*	SE	χ^2	*p*
FIV	58	0.52	0.47	1.2	0.3	–0.43	0.48	0.79	0.37
FeLV	58	0.57	0.48	1.4	0.2	–0.42	0.48	0.77	0.34
FCV	60	0.64	0.29	5.0	0.026*	–0.14	0.29	0.2	0.6
FPV	60	0.63	0.30	4.3	0.037*	0.46	0.30	0.23	0.1

Note: *B* is the parameter estimate in the generalized linear model, SE is standard error, and χ^2 is Wald's statistics. Interactions of the effects were insignificant in all models. FIV, feline immunodeficiency virus; FeLV, feline leukemia virus; FCV, feline calicivirus; FPV, feline panleukopenia virus.

*Significant differences are between the serum prevalence in the village and at the herdsmen stations (generalized linear model: $p < 0.05$).

herdsmen stations could be more territorial and aggressive than those in the village, and therefore, a higher frequency of agonistic contacts between animals and a greater risk of being bitten (the main route of viral transmission) led to an increased risk of FIV infection. At the same time, in contrast to other studies (Fromont et al. 1997; Courchamp et al. 1998) and to our expectations, the seroprevalence of FIV did not predominate in males, with four of six positive animals being female. This may suggest that FIV could also be transferred through sexual contact as an alternative route in the study area.

However, it is difficult to explain the same tendency for the FeLV seroprevalence. According to other observations (Fromont et al. 1997, 1998), FIV and FeLV have opposite transmission strategies and FeLV is more prevalent among socially active cats. Thus, we would expect more FeLV-positive cats in the village where population density is higher than at the stations. However, our results do not support this hypothesis, as there was no significant difference between the seroprevalence of FeLV in high- and low-density sites. The seroprevalence in our study was higher than that of other studies (Danner et al. 2007; Dubey et al. 2009; Gleich et al. 2009) and there may be additional unexamined risk factors that influence patterns of FeLV seroprevalence in the study area such as phenotype, body mass, physical condition, and kinship relations, any of which might be hidden by the density factor.

Interspecific differences in the seroprevalence

As we supposed, the prevalence of viruses (FCV, FPV, FIV, and FeLV) were lower in Pallas' cats than in domestic cats. In Pallas' cats, we found no evidence of infection with viruses that depended significantly on population density (FCV and FPV) and found low prevalence of FIV and FeLV (about 5% of each) that are transmitted through sexual or aggressive contacts. On the one hand, these results suppose that domestic cats might be a source of FIV and FeLV for Pallas' cats. An observation that might be consistent with this was the slightly higher seroprevalence of these viruses in domestic cats living at the herdsmen stations. These territories (including abandoned herdsmen stations) are visited by Pallas' cats for hunting, breeding kittens, and as shelter (based on our visual observations). Therefore, there is the possibility of interspecific contacts in the study area. On the other hand, it is unlikely we did not find any Pallas' cats that were positive for FCV given the high prevalence of FCV in domestic cats, as well as potential aggressive and (or) sexual interspecific contacts which are necessary for the transmission of FIV and FeLV. Based on this, we believe that it is more likely that the rate of interspecific contacts is very low in the study area. Transmission of FIV and FeLV mainly occurs within a species. Comparatively, low population density of both cat species and specific differences in spatial organization (e.g., large home range in Pallas' cats) allow Pallas' cats to avoid both inter- and intra-specific direct contacts. Therefore, the risk of being infected by FIV, FeLV, and FCV is low, as is the spread of these viruses in the population of Pallas' cats.

The lack of FPV exposure in Pallas' cats was harder to explain than FCV. FPV infects the most members of the family Felidae, including Pallas' cats (Quesenberry 1984; Hofmann-Lehmann et al. 1996; Steinel et al. 2001; Ketz-Riley et al. 2003). The virus has high resistance in the environment and indirect transmission through feces and feces-contaminated vomites is largely predominant (Reif 1976). We supposed that the prevalence of FPV would be lower in Pallas' cats than in domestic cats in the study area, because Pallas' cats have larger home ranges and lower population densities, and accordingly, there is less opportunity to have contact with infected areas compared with domestic cats that are housed close together. The population density of both species in our study area is comparatively low, which may decrease the possibility of FPV infection in Pallas'cats, but does not entirely exclude it. One explanation for the total lack of FPV-positive Pallas' cats in our study could be the high mortality rate of young Pallas' cats in the study area. However, our data are not sufficient to support this supposition, except for some anecdotal cases (Kirilyuk and Puzanskii 2000). Additionally, optimal conditions for the persistence of FPV in the environment (4–25 °C) are limited to a short period of a year (actually there are few days per year when the air temperature does not fall outside these limits), which may also reduce the risk of infection for wild Pallas' cats.

Theoretically, the interspecific differences might also be determined by technical reasons, i.e., the different sampling time and the small sample size of Pallas' cats. Concerning differences in the sampling time, we sampled Pallas' cats in 2011–2014 (February–March) and domestic cats in 2013 (February–March; October–November) (Table 1). According to owner surveys, there were no remarkable changes in cat mortality during the study period. Additionally, antibodies of the tested viruses may persist for months (up to 1 year). It is unlikely that the patterns of seroprevalence of different tested viruses are the result of contact by domestic cats with viruses at the same time. Therefore, we most probably obtained an average picture of the seroprevalence over the period of 2011–2014 when the Pallas' cats were sampled as well and could contact with the viruses (Naidenko et al. 2014). We also suggest that the small sample size of Pallas' cats (24 animals) is quite reliable, because it allows the detection of antibodies against viruses (FIV and FeLV) with lower transmissibility, virulence, and therefore, lower prevalence than FCV and FPV. Thus, in our opinion, the influence of these technical factors has been minimal and our data reflect natural patterns of viral seroprevalence in the two cat species in the study area.

Conclusions

Our results indicate that four of five viruses are circulating in the study area, with the exception of FHV. The pattern of FCV and FPV prevalence differed from FIV and FeLV. FCV and FPV seroprevalence did not depend on sex and predominated in the group of cats living closely together in the village. The prevalence of FIV and FeLV were similar in areas with different population density

of domestic cats. The patterns of seroprevalence between species testify to the low rate of interspecific contacts. In Pallas' cats, we only found evidence of exposure to the viruses that demand close direct contacts between animals (mainly aggressive and (or) sexual), typical in the breeding season. It is possible that severe, arid climatic features (low humidity, extremely high or low temperatures) may also reduce patterns of viral prevalence in study area, decreasing the risk of infection for both cat species. Future research should be directed at the estimation of other risk factors of tested viruses in domestic cats, such as phenotype, body mass, physical condition, and kinship relations that might be hidden by the density factor. Also our results emphasize the need to conduct studies that analyze the spatial organization of both domestic cats and Pallas' cats to identify the natural character of their relations.

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