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ORIGINAL RESEARCH

Incidence of Chlamydia spp., FIV, FeLV in Free-Roaming Cats in Slovakia

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Purpose: Free-roaming cats represent a potential reservoir of infectious diseases. The most common co-infections of free-roaming cats include mixed viral, bacterial, fungal, yeast and parasitic infections. This study focuses on the occurrence of *Chlamydia* spp. feline immunodeficiency virus (FIV), feline leukaemia virus (FeLV) and their co-infections. The diseases accompanied by immune suppression, such as FIV, create favourable conditions for the onset of other diseases and co-infections. The result of co-infection may be a higher susceptibility for other pathogens, as well as the occurrence of more severe clinical symptoms.

Patients and Methods: The study involved 168 (113♀ and 55♂) free-roaming adult cats during the years 2021–2022. All cats belonged to Slovak citizens with permanent residence in the Slovak Republic. Blood samples and swabs (Invasive EUROTUBO® Collection sterile swab, Deltalab O8191 Rubí, Spain) from the conjunctival sac were taken from 168 cats to be later tested by PCR and ELISA methods. Statistical analysis was also performed.

Results: The overall prevalence of *Chlamydia* spp. was 17.26%, of FIV 15.48%, and 5.95% of FeLV. The most significant finding in our study was 3.57% co-infection of FIV and *Chlamydia* spp. in tested cats.

Conclusion: The observed prevalence of *Chlamydia* spp. FIV and FeLV indicates that the presence of these pathogens in populations of free-roaming cats is endemic.

Keywords: cats, chlamydiosis, FIV, FeLV

Introduction

Free-roaming cats are a potential reservoir of infectious diseases. Pathogens with zoonotic potential include *Chlamydia psittaci* (*C. psittaci*) and *Chlamydia felis* (*C. felis*); since there were not many confirmed human infections transmitted from free-roaming cats, our study focuses on the significant impact of these pathogens on the health of cats. Even though evidence of transmission of *C. felis* to humans exists, at the moment there is no epidemiological proof of *C. felis* representing a high zoonotic risk. For example, *Chlamydia* isolated from the conjunctiva of a patient infected by human immunodeficiency virus (HIV) was identical with *C. felis* isolated from cats.[1] *C. felis* may induce conjunctivitis of humans who are in close contact with infected cats.[2–4]

Feline chlamydiosis caused by the bacteria known as *C. felis* impacts the upper respiratory tract and the conjunctivas of cats. The incubation period usually lasts for 2 to 5 days. Clinical signs have often been shown to initially affect one eye, subsequently extending to both eyes. The infection is accompanied by conjunctivitis with extreme hyperaemia of the third eyelid and blepharospasm.[3,5] Ocular discharge observed in feline chlamydiosis cases is watery at first, in more severe cases the discharge may later become mucous or mucopurulent. Chemosis of conjunctiva may also develop. *C. felis* has also been shown to latently survive in the gastrointestinal and reproductive tracts of cats. Pathogenesis and pathogenicity of particular *Chlamydia* species vary, but the causes of the variation in the pathogenesis are not yet known. Conjunctivitis and respiratory infection of cats are more frequent in young stray cats.[6,7] Most cats infected by *C. felis* cease to excrete *C. felis* from the conjunctiva 60 days post infection; however, in some cats, a latent infection may

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persist.[8,9] Chlamydial conjunctivitis is the second most common conjunctivitis in cats after herpes viral conjunctivitis. Kittens infected by *C. psittaci* may show various symptoms such as increased body temperature, lethargy, weight loss,[10] conjunctivitis and rhinitis.[11]

Feline immunodeficiency virus (FIV), which was isolated in 1986, belongs to the family *Retroviridae*, and the genus *Lentivirus*.[12–14] It causes chronic and even fatal disease of cats. The occurrence of the infection significantly increases with age, especially in free-roaming male cats.[15–17] The most common transmission of FIV occurs when adult infected cats bite or scratch other cats. Cats in the acute stage of infection show non-specific symptoms such as anaemia, gingivitis, uveitis, conjunctivitis, pyoderma, depression and diarrhea. The infection may be present without clinical signs for months or even years.[18] The complex of medical complications is similar to AIDS of humans characterized by chronic infections, affecting primarily the oral cavity, the respiratory system and less commonly the digestive tract.[15,17] The terminal stage of progress from 6 to 12 months leads to death as a result of complete innate immune system failure.[18,19] Apart from FIV being studied in the context of cat health, the similarities to HIV infection and disease, FIV has provided a useful model for in vitro as well as in vivo studies.[20,21]

Feline leukemia virus (FeLV) is a gamma retrovirus first described in 1964 at the University of Glasgow by Jarrett et al.[22] FeLV is commonly transmitted through saliva; therefore, mutual grooming of cats, nose-to-nose contact, and shared food and water bowls can become sources of infection.[23] Concentration of the virus in saliva is higher than in plasma, and the virus is also transmitted through faeces,[3] milk and urine.[15] Viral leukaemia complex includes lympho-proliferative disorders and organ cancers, disruption of haematopoiesis and immune deficiency.[18] Progress of the disease and clinical signs depends on the immune status of infected cats, age as well as on the pathogenicity, virulence, and titre of the virus.[24] Since 1980, the seroprevalence of FeLV associated with ongoing testing and vaccination programs has significantly decreased in several countries.[25] In contrast to FeLV, the first isolation of feline immunodeficiency virus (FIV) was carried out in 1986 from two laboratory cats infected with a suspension of tissue, originated from stray cats in Petaluma, California. The isolated virus had been called T-lymphotropic virus at that time, and the virus was later renamed FIV. In 1987, sequencing of the viral genome confirmed that the isolates (FIV-Petaluma, FIV-Pet) were lentiviruses.[26]

The most common cases of co-infection in free-roaming cats involve viral, bacterial, and fungal pathogens, including yeasts and parasites.[27] Our study aimed to assess the risk factors of Chlamydia spp., FIV and FeLV infections and co-infections in cats in the selected area in the district of Košice, Slovakia.

Materials and Methods

Examination of Cats and Sampling

Cats included in our study were divided into categories according to their sex, the location of sampling (veterinary ambulance, field), and whether they were kept outdoors, or both indoors and outdoors ("indoor-outdoor" cats). We categorized the cats based on their housing conditions and the evaluation of breeding conditions similarly to Candela et al.[28] Every cat categorized as free-roaming had an owner and access to both indoor and outdoor areas. The term "free-roaming cats" was used to describe cats with owners and free to roam outside. All cats belonged to Slovak citizens with permanent residence in the Slovak Republic, or were sampled on the state's territory. Sampled cats were either European shorthair cats or crossbred from this breed. None of the cats included in our study were castrated. Also, none of the cats had been vaccinated prior to sampling. The cats examined in the study were treated in accordance with the rules of good animal practice. Informed consent with clinical examination was obtained from each owner of a free-roaming cat. In 2021–2022, blood samples and swabs (Invasive EUROTUBO® Collection sterile swab, Deltalab O8191 Rubí, Spain) from the conjunctival sac were taken from 168 free-roaming cats. They were of both sexes and of various ages. In a number of cases, the age of the cats was estimated according to their teeth. The free-roaming cats tested in our study had not been vaccinated against FIV, FeLV or chlamydiosis. Both clinically healthy cats and cats displaying clinical signs of disease were sampled.

PCR Examination for Chlamydiosis

In the years 2021–2022, we examined 168 cats for the presence of *Chlamydia* spp. in samples from the lower conjunctival sac swabs by PCR. To detect *Chlamydia* spp., samples were taken by means of a deep swab from the lower conjunctival sac after the application of local anaesthetic with oxybuprocain hydrochloride as active substance (Benoxi 0.4% int opo, Unimed Pharma Ltd., Slovak Republic). The swab with the sample was stirred in 300 µL of sterile physiological saline solution. Paired samples were labelled providing the relevant identification details (date, sampling location, identification signs of the animal) and stored at −80°C until examination. We conducted DNA isolation according to the spin protocol for DNA purification using a commercial isolation kit DNeasy® Blood & Tissue kit (QIAGEN®, Germany). *Chlamydia* spp. detection was carried out using conventional PCR according to Halánová et al.[7]

Serological Examination

We examined the collected cat blood samples for the presence of FIV antibodies and FeLV antigen using the SNAP FIV/FeLV Combo Test. To detect FIV antibodies and FeLV antigen, we took blood from vena cephalica antebrachii. We chose the vena jugularis to collect blood from cats sedated with medetomidinum (Narcostart 1mg/mL inj., LeVet Beheer, B.V). The obtained full blood containing anticoagulant (K_3EDTA) was tested for FIV/FeLV using commercially available ELISA tests (SNAP FIV/FeLV Combo Test, IDEXX Laboratories). Confirmatory testing was not performed due to satisfactory sensitivity and specificity of the commercial kits used for the screening.

Statistical Analysis

Statistical analyses were performed on the statistical analysis software GraphPad Prism, version 5.01 (GraphPad Software, Inc., San Diego, California, USA). The differences in prevalence of FIV, FeLV and *Chlamydia* spp. in cats between both sexes and each area (ie, rural, urban and suburban areas) were tested by the chi-square (χ^2) test or the Fisher's exact test, and p values of less than 0.05 were considered significant. Besides the prevalence of the occurrence of *C. felis*, we calculated the relative risk using standard methodology, ie by calculating the relative risk of the determination of the confidence interval using the EpiInfo 2023. Relative risks were calculated for individual disease and were categorized by symptoms, method of keeping cats, and sex.

Results

The overall prevalence of *Chlamydia* spp. was 17.26%, of FIV 15.48%, and 5.95% of FeLV. The most significant finding in our study was 3.57% co-infection of FIV and *Chlamydia* spp. in tested cats. A total of 168 cats (55 males, 113 females) from three areas, ie, rural, urban and suburban areas, were evaluated (Tables 1–5, Figure 1). Different sex compositions were shown for each area, but tested differences among three areas were statistically non-significant ($\chi^2 = 5.037$, df = 2, p = 0.0806). We try to compare differences in the prevalence of FIV, FeLV, and *Chlamydia* spp. antigens between sexes in examined material of three areas. Nevertheless, no significant differences between males and females by using Fisher's exact test were observed in this study (Table 6 with exception of FeLV antigens in urban areas (p = 0.039, p < 0.05)). Likewise, a statistically non-significant difference (p = 0.6140) between the sexes was confirmed when evaluated without regard to infection and areas. A map with the areas indicated is included as Figure 2.

The most frequent clinical symptoms of disease diagnosed in *Chlamydia* spp. positive cats were conjunctivitis (24/29), ocular discharge (23/29), pyrexia, anorexia, upper respiratory tract infection (10/29), and blepharospasm (9/29). It can be stated that conjunctivitis and ocular discharge, pyrexia, anorexia, upper respiratory tract infection are usually accompanied by symptoms of *Chlamydia* spp. disease.

Our calculation of relative risk showed that diarrhea symptoms and other factors such as sex, place of capture (city, suburban area and rural area) are statistically insignificant for *Chlamydia* spp. disease. On the other hand, we proved that conjunctivitis (RR: 23.0, SE: 0.45, CI 9.52 to 55.6), ocular discharge (RR: 19.2, SE: 0.41; CI 8.56 to 42.9) and blepharospasm (RR: 7.95; SE: 0.21, CI 5.26 to 12.0) are statistically significant symptoms for *Chlamydia* spp. disease.

Table 1 Free-Roaming Cats Examined for FIV, FeLV and Chlamydia Spp. in Rural Areas, Divided by Sex, the Place of Trapping and Sampling

Cat Examined in Rural Areas	Females (♀)				Males (♂)				Total
	i-o[#]		o[#]		i-o		o		
	VA[#]	F[#]	VA	F	VA	F	VA	F	
With clinical symptoms	0	3	0	0	0	1	0	0	4
Chlamydia spp. +	0	2	0	2	0	0	0	0	4
FIV/*Chlamydia* spp. +	0	0	0	2	0	0	0	0	2
Without clinical symptoms	1	7	1	8	0	6	1	0	24
FIV +	0	0	0	0	0	1	0	1	2
FIV/FeLV +	0	1	0	0	0	0	0	0	1
Chlamydia spp. +	0	0	0	0	0	1	0	0	1
Total	1	13	1	12	0	9	1	1	38
Number of examined cats i-o/o	14		13		9		2		
Number of examined ♀ and ♂	27				11				
Number of all examined cats	38								

Notes: [#]Indoor-outdoor cats (i-o); outdoor cats (o); veterinary ambulance (VA); fieldwork (F).

Table 2 Free-Roaming Cats Examined for FIV, FeLV and Chlamydia Spp. in Urban Areas, Divided by Sex, the Place of Trapping and Sampling

Cat Examined in Urban Areas	Females (♀)				Males (♂)				Total
	i-o[#]		o[#]		i-o		o		
	VA[#]	F[#]	VA	F	VA	F	VA	F	
With clinical symptoms	0	0	0	0	0	0	0	0	0
FIV/FeLV +	0	0	1	1	0	0	1	1	4
Chlamydia spp. +	0	0	7	1	1	0	2	0	11
FIV/*Chlamydia* spp. +	0	0	2	1	0	0	0	0	3
FIV/FeLV/ *Chlamydia* spp. +	1	0	0	0	0	0	0	0	1
Without clinical symptoms	0	35	0	15	0	6	0	6	62
FIV +	0	2	1	0	0	2	4	0	9
FeLV +	0	0	0	0	0	0	3	0	3
Chlamydia spp. +	0	0	1	0	0	0	1	0	2
Total	1	37	12	18	1	8	11	7	95
Number of examined i-o/o	38		30		9		18		
Number of examined ♀ a ♂	68				27				
Number of all examined cats	95								

Notes: [#]Indoor-outdoor cats (i-o); outdoor cats (o); veterinary ambulance (VA); fieldwork (F).

Table 3 Free-Roaming Cats Examined for FIV, FeLV and Chlamydia Spp. in Suburban Areas, Divided by Sex, the Place of Trapping and Sampling

Cat Examined in Suburban Areas	Females (♀)				Males (♂)				Total
	i-o[#]		o[#]		i-o		o		
	VA[#]	F[#]	VA	F	VA	F	VA	F	
With clinical symptoms	0	0	0	0	0	0	1	0	1
FIV +	0	0	0	0	0	0	2	0	2
Chlamydia spp. +.	0	2	0	0	0	0	0	0	2
FIV/*Chlamydia* spp. +	1	0	0	0	0	0	0	0	1
FIV/FeLV/ *Chlamydia* spp. +	1	0	0	0	0	0	0	0	1
Without clinical symptoms	1	3	2	8	2	8	0	3	27
Chlamydia spp. +	0	0	0	0	0	0	1	0	1
Total	3	5	2	8	2	8	4	3	35
Number of examined i-o/o	8		10		10		7		
Number of examined ♀ a ♂	18				17				
Number of all examined cats	35								

Notes: [#]Indoor-outdoor cats (i-o); outdoor cats (o); veterinary ambulance (VS); fieldwork (F).

Table 4 Number of Positive Cats for FIV, FeLV and Chlamydia Spp. in Particular for Rural Areas, Urban Areas and Suburbs. This Table Does Not Take Co-Infections into Account

	Rural Areas	Urban Areas	Suburban Areas	TOTAL
Number of examined cats	38 27♀, 11♂	95 68♀, 27♂	35 18♀, 17♂	168 113♀ (67.26)[#] 55♂ (32.74)[#]
Number of FIV positive cats	5 (13.16)[#] 3♀, 2♂	17 (17.89)[#] 9♀, 8♂	4 (11.43)[#] 2♀, 2♂	26 (15.48)[#] 14♀ (12.39)[#] 12♂ (21.82)[#]
Number of FeLV positive cats	1 (2.63)[#] 1♀, 0♂	8 (8.42)[#] 3♀, 5♂	1 (2.86)[#] 1♀, 0♂	10 (5.95)[#] 5♀ (4.42)[#] 5♂ (9.09)[#]
Number of *Chlamydia* spp. positive cats	7 (18.42)[#] 6♀, 1♂	17 (17.89)[#] 13♀, 4♂	5 (14.29)[#] 4♀, 1♂	29 (17.26)[#] 23♀ (20.35)[#] 6♂ (10.91)[#]

Notes: [#]Expressed as a number and percentage (bracketed); male (♂), female (♀).

Rural Areas

Samples from 38 cats (27♀, 11♂) were collected in rural areas (Table 1). Out of 10 cats displaying clinical symptoms of infectious diseases, 4 were diagnosed without laboratory confirmation. A total of 28 cats showed no clinical symptoms of disease. FIV was confirmed in 2 asymptomatic cats. Asymptomatic co-infection of FIV/FeLV was confirmed in 1 cat. Laboratory examinations confirmed the presence of *Chlamydia* spp. in a sample from one cat with no clinical symptoms of disease.

Table 5 Number of Cats Infected by FIV, FeLV and Chlamydia Spp. and Co-Infections FIV/Chlamydia Spp., FeLV/Chlamydia Spp., FIV/FeLV/ Chlamydia Spp. and FIV/FeLV

	Rural Areas	Urban Areas	Suburban Areas	TOTAL
Examined cats	38	95	35	168
FIV positive cats	2 (5.26)[#]	9 (9.47)[#]	2 (5.71)[#]	13 (7.74)[#]
FeLV positive cats	0 (0.00)[#]	3 (3.16)[#]	0 (0.00)[#]	3 (1.79)[#]
Chlamydia spp. positive cats	5 (13.16)[#]	13 (13.68)[#]	3 (8.57)[#]	21 (12.50)[#]
FIV/*Chlamydia* spp. positive cats	2 (5.26)[#]	3 (3.16)[#]	1 (2.86)[#]	6 (3.57)[#]
FeLV/*Chlamydia* spp. positive cats	0 (0.00)[#]	0 (0.00)[#]	0 (0.00)[#]	0 (0.00)[#]
FIV/FeLV/*Chlamydia* spp. positive cats	0 (0.00)[#]	1 (1.05)[#]	1 (2.86)[#]	2 (1.19)[#]
FIV/FeLV positive cats	1 (2.63)[#]	4 (4.21)[#]	0 (0.00)[#]	5 (2.98)[#]
TOTAL	10	33	7	50

Notes: [#]Expressed as a number and percentage (bracketed).

Laboratory examinations confirmed *Chlamydia* spp. disease in 4 cats, which displayed symptoms such as conjunctivitis (4/4), ocular discharge (4/4), blepharospasm (2/4), chemosis (1/4).

Co-infection of FIV/*Chlamydia* spp. was recorded in 2 cats displaying pyrexia, anorexia, apathy, gingivitis, infection of upper respiratory tract (2/2), conjunctivitis, ocular discharge (2/2), infection of lower respiratory tract, blepharospasm (1/2).

Urban Areas

We tested 95 cats (68♀, 27♂) in the city of Košice, Slovakia (Table 2). Clinical symptoms were observed in 19 cats. All cats showing clinical signs of an infectious disease tested positive for at least one of our target diseases.

Four cats displaying infectious disease symptoms different than symptoms typical for our target diseases then tested positive for FIV/FeLV. We have observed anorexia and diarrhea (4/4), cachexia and apathy (2/4), abscesses and lymphadenopathy (1/4). These cats were judged as co-infected with FIV/FeLV.

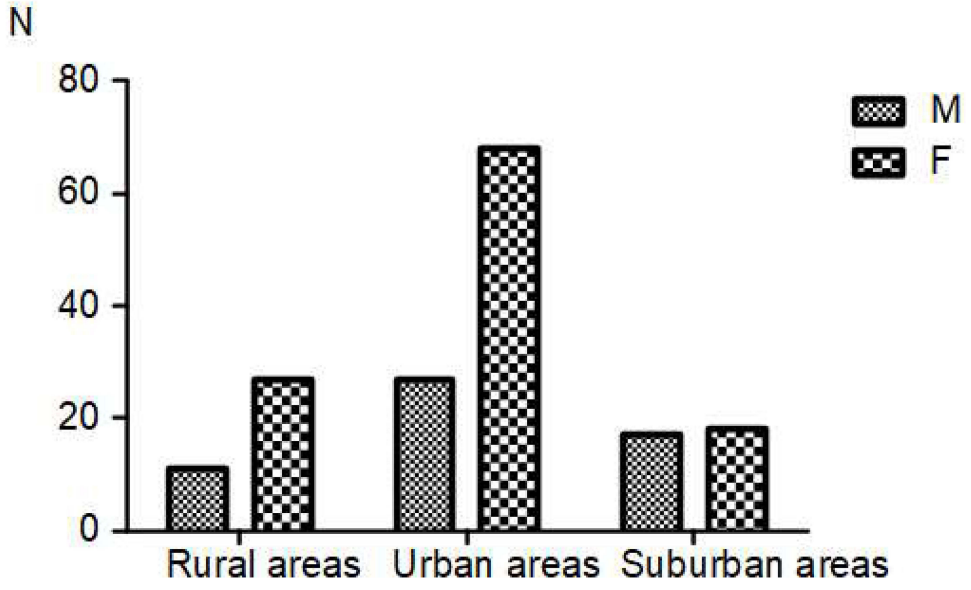

Figure 1 Numbers of cats examined in each area.

Table 6 Fisher's Exact Test Results for Comparison of FIV, FeLV, and Chlamydia Spp. Detected for Each Area

	FIV	FeLV	*Chlamydia* spp.
Rural areas	0.6154	0.5007	0.6482
Urban areas	0.0772	0.0393*	0.7708
Suburban areas	1.000	1.000	0.3377
Regardless of the areas	0.1187	0.2985	0,1909

Note: *Statistically significant results.

Chlamydia spp. were observed in 11 cats with clinical symptoms. We observed various symptoms of Chlamydia infection, as follows: conjunctivitis (11/11), ocular discharge (9/11), blepharospasm, infection of upper respiratory tract (5/11), chemosis, pyrexia, anorexia, cachexia (2/11), infection of lower respiratory tract, lymphadenopathy, apathy (1/11) in cats which were positive for *Chlamydia* spp. A large group of these cats were young cats whose age was estimated to be around one year.

Co-infection of FIV/*Chlamydia* spp. was detected in 3 cats. FIV-positive cats showed noticeable symptoms such as pyrexia, anorexia, gingivitis and stomatitis, abscesses, conjunctivitis, ocular discharge (3/3), cachexia (2/3), apathy (1/3). In this group of cats, poor hair quality was also detected, with subsequent ectoparasitic infection confirmed microscopically (*Notoedres cati*). Abscesses in the neck area were also present in these cats. All cats with clinical symptoms of FIV came from the same location (a housing estate).

The remaining 62 cats showed no clinical symptoms of diseases. Laboratory tests for these cats were negative for all of our target diseases. Asymptomatic disease was confirmed in 14/62 cats. FIV was confirmed in 9/14 cases, FeLV in 3 cases and *Chlamydia* spp. in two remaining cases.

We detected a co-infection of FIV/FeLV/*Chlamydia* spp. in one cat. The cat was in the terminal stage of the disease (anorexia, apathy, uveitis, respiratory and gastrointestinal symptoms).

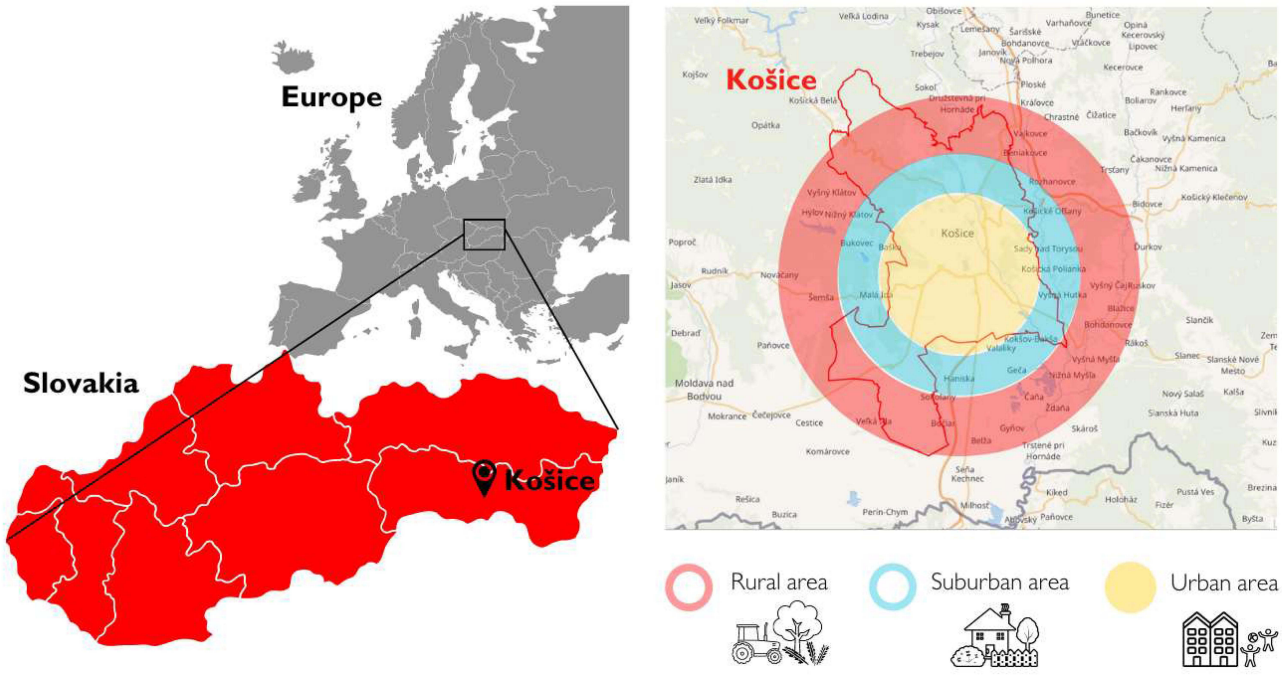

Figure 2 Map of Košice with urban, suburban and rural areas indicated.

Suburban Areas

Suburban area is defined as the territory between rural and urban; in our study suburban areas were characterized by the presence of residential neighborhoods with houses with large backyards. In suburban areas, we tested a total of 35 cats (18♀, 17♂) (Table 3). We recorded 7 cats with clinical symptoms of the disease in suburban areas. One male cat, despite showing clinical symptoms of an infectious disease, did not test positive for any of our target diseases. We recorded 28 cats with no clinical symptoms. In one case, an asymptomatic outdoor male cat tested positive for *Chlamydia* spp. The cat was brought to the veterinary ambulance for a preventive check-up. Laboratory examinations confirmed presence of FIV in 2 cats displaying pyrexia, anorexia, diarrhea, apathy, gingivitis and stomatitis, infection of upper respiratory tract (2/2), and infection of lower respiratory tract (1/2), cachexia (1/2). Two female cats sampled in a field were positive for *Chlamydia* spp. In both the *Chlamydia* spp. positive cats, conjunctivitis and ocular discharge were confirmed. In one indoor-outdoor female cat, examined in the veterinary ambulance, co-infection of FIV/*Chlamydia* spp. was confirmed. The cat positive for co-infection of FIV/*Chlamydia* spp. showed apathy, anorexia, cachexia and also increased body temperature, gingivitis, diarrhea, infection of upper respiratory tract, conjunctivitis and ocular discharge. We confirmed one case of co-infection of *Chlamydia* spp./FIV/FeLV in one female cat; the cat was in the terminal stage of disease.

Prevalence of Diseases

Prevalence of individual diseases and their co-infections are concluded in Tables 4 and 5. Table 4 shows the number of recorded infections with FIV, FeLV and *Chlamydia* spp. in individual areas along with prevalence. Prevalence is calculated for both sexes and total prevalence is also calculated. Table 5 shows the number of cats with infections and co-infections in the respective areas.

The Risk of Infection of Chlamydia spp., FIV and FeLV in Cats Kept in Urban, Suburban and Rural Areas

In our study, the probability of disease caused by *Chlamydia spp*. was 13.93 times higher in free-roaming cats with clinical symptoms in comparison with free-roaming cats without clinical symptoms of disease. Tables 7–9 present relative risks for *Chlamydia* spp., FIV and FeLV, respectively. Table 10 shows the influence of individual diseases on other diseases and also interrelationships between the diseases expressed by relative risk.

No relationship between the occurrence of the diseases of our interest, the location of cats, or the general occurrence of diseases together was confirmed.

Our calculation of the relative risk by determination of the confidence interval showed that blepharospasm and chemosis, along with sex of the captured cats and the areas where they were captured (urban, suburban and rural area), are statistically insignificant for FIV disease. On the other hand, we proved that gingivitis and stomatitis (RR: 11.1, SE: 0.25; CI 6.74 to 18.4), anorexia (RR: 9.72, SE: 0.30, CI 5.34 to 17.7), diarrhea (RR: 8.36 SE: 0.25, CI 5.09 to 13.8), are statistically significant symptoms for FIV disease.

Discussion

Analysis of Chlamydiosis

The *Chlamydia* spp. disease of cats was registered in Slovakia for the first time in 2001. A study based on occurrence of *C. felis* in cats without reaction to broad spectrum 7-day antibiotic therapy was performed a year later. The disease was diagnosed by direct antigen test and confirmed in 10 out of 13 clinically ill cats.[29] Several studies focused on the prevalence of *C. felis*. *Chlamydia* infections are endemically widespread among domestic cats. The occurrence of *Chlamydia* spp. is worldwide.[7]

Surveys of data on prevalence *Chlamydia* spp. in Slovakia and other countries were obtained from selected research works: in Italy, 20% prevalence was reported,[30] in Japan, 59.1%,[2] in Australia 11.5%,[31] in Slovakia, 16.6%,[32] in the USA, 4.6%,[33] in Slovenia 16.7%,[34] 19.1% in stray cats in Switzerland,[3] 12.1% in China,[35] and the prevalence in our study was 17.26% (29 cats).

Table 7 The Calculation of Relative Risk During the Disease of FIV

Statistical/ Observed Symptom	Pyrexia	Ano rexia	Cachexia	Diarr hoea	Apathy	Gingivitis and Stomatitis	Absce sses (neck, ears)	Lymphade nopathy	Upper Respiratory Tract Disease	Lower Respi ratory Tract Disease	Conjun ctivitis	Ocular Discharge	Blepha rospasm	Chem osis	Male	Out door	Urban area	Rural area	Sub urban area
#RR	6.88	9.72	5.78	8.36	7.02	11.1	7.45	5.35	3.76	4.18	2.13	2.22	1.47	1.64	1.76	2.13	1.23	1.03	0.69
#SE	0.29	0.30	0.30	0.25	0.27	0.25	0.20	0.35	0.35	0.38	0.37	0.37	0.65	0.89	0.36	0.38	0.37	0.43	0.51
#CIL	3.87	5.34	3.19	5.09	4.10	6.74	5.04	2.70	1.89	1.97	1.02	1.07	0.41*	0.29*	0.87*	1.00	0.59*	0.44*	0.25**
#CIH	12.2	17.7	10.5	13.8	12.0	18.4	11.0	10.6	7.49	8.89	4.44	4.62	5.32*	9.40*	3.56*	4.52	2.56*	2.38*	1.89*

Note: *Insignificant statistics.

Abbreviations: #RR-Relative risk, SE-Standard error, CIL-Lower value of interval CI, CIH-Upper value of interval CI.

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Table 8 The Calculation of Relative Risk During the Disease of FeLV

Statistical/ Observed Symptom	Pyrexia	Ano rexia	Cach exia	Diar rhoea	Apathy	Gingivitis and Stomatitis	Abscesses (neck, ears)	Lympha denopathy	Upper Respiratory Tract Disease	Lower Respiratory Tract Disease	Conjun ctivitis	Ocular Discharge	Blepha rospasm	Chem osis	Male	Out door	Urban Area	Rural Area	Suburban Area
RR[#]	2.75	12.5	8.67	23.7	3.95	8.67	4.56	17.6	2.55	5.75	1.20	1.25	1.96	4.56	2.05	2.63	1.79	0.86	0.42
SE[#]	0.74	0.60	0.57	0.56	0.72	0.57	0.92	0.47	0.74	0.69	0.76	0.76	1.00	0.92	0.61	0.67	0.67	0.77	1.04
CIL[#]	0.64*	3.86	2.81	7.90	0.95*	2.81	0.74*	6.97	0.59*	1.48	0.27*	0.28*	0.27*	0.74*	0.62*	0.70*	0.48*	0.19*	0.05*
CIH[#]	11.8*	40.4	26.8	71.1	16.3*	26.8	28.2*	44.3	11.0*	22.4	5.40*	5.63*	14.0*	28.2*	6.85*	9.90*	6.75*	3.89*	3.26*

Note: *Insignificant statistics.

RR, Relative risk; SE, Standard error; CIL, Lower value of interval CI; CIH, Upper value of interval CI.

Table 9 The Calculation of Relative Risk During the Chlamydia Spp. Infection

Statistical/ observed Symptom	Pyrexia	Ano rexia	Cach exia	Diarrhoea	Apathy	Gingivitis and Stomatitis	Absce sses (neck, ears)	Lymphade nopathy	Upper Respiratory Tract Disease	Lower Respiratory Tract Disease	Conjun ctivitis	Ocular Discharge	Blepha rospasm	Chem osis	Male	Out door	Urban Area	Rural Area	Sub urban Area
#RR	5.79	4.39	4.14	1.82	5.03	4.95	4.73	4.73	5.37	3.68	23.0	19.2	7.95	6.56	0.54	2.50	1.09	1.09	0.79
#SE	0.27	0.30	0.31	0.52	0.29	0.29	0.34	0.34	0.28	0.38	0.45	0.41	0.21	0.18	0.43	0.37	0.34	0.39	0.45
#CIL	3.38	2.42	2.23	0.66*	2.86	2.81	2.42	2.42	3.08	1.76	9.52	8.56	5.26	4.56	0.23*	1.21	0.55*	0.50*	0.32*
#CIH	9.92	7.94	7.68	5.04*	8.84	8.74	9.25	9.25	9.36	7.72	55.6	42.9	12.0	9.43	1.25*	5.20	2.14*	2.36*	1.94*

Note: *Insignificant statistics.

Abbreviations: #RR, Relative risk; SE, Standard error; CIL, Lower value of interval CI; CIH, Upper value of interval CI.

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Table 10 The Calculation of Relative Risk, Standard Error, Lower Interval Values CI, Upper Interval Value CI for Chlamydia Spp., FIV and FeLV

The Risk of Observed Infection	The Risk of *Chlamydia* Spp. Infection in FIV Positive Cats	The Risk of *Chlamydia* Spp. Infection in FeLV Positive Cats	The Risk of *Chlamydia* spp. Infection in FIV and FeLV Positive Cats	The Risk of FIV Infection in *Chlamydia* Spp. Positive Cats	The Risk of FIV Infection in FeLV Positive Cats	The Risk of FIV and FeLV Infection in *Chlamydia* spp. Positive Cats	The risk of Infection FIV or FeLV or *Chlamydia* spp. or Co-Infections in Cats Brought to Veterinary Ambulance	The risk of infection FIV or FeLV or *Chlamydia* spp. or Co-Infections in Cats Caught in Field
#RR	2.13	1.2	1.9	2.1	1.2	1.8	7.9	0.43
#SE	0.37	0.8	0.8	0.4	0.7	0.4	0.3	0.18
#CIL	1.02	0.3*	0.4*	1	0.3*	0.9*	4	0.31*
#CIH	4.44	5.4*	9.5*	4.2	4.3*	3.7*	15	0.61*

Note: *Insignificant statistics.
Abbreviations: #RR, Relative risk; SE, Standard error; CIL, Lower value of interval CI; CIH, Upper value of interval CI.

In Slovakia and other countries, prevalence of *C. felis* in healthy domestic cats was reported as follows: Italy 3.3%[36] USA 0%,[33] Slovakia 6.45%,[32] our research 2.38% (4 cats).

Studies in several countries worldwide calculated the following prevalence of *C. felis* in cats showing clinical symptoms: in Slovakia, 42.1% in the population of cats from animal shelters, in Slovakia, 42.9% in the stray cat population,[32] in the USA, 0% in the category of cats living in animal shelters or breeding farms,[37] in Europe 10%,[5] in Romania 65.3%,[38] and in our research, 69.44% (25 cats). The prevalence of *C. felis* in healthy domestic cats in our study was 2.38%, which is consistent with results of *C. felis* prevalence in Italy. Similarly, our prevalence result in cats exhibiting clinical symptoms has a significantly high prevalence rate comparable with the study from Romania.[38]

Data of prevalence observed in the individual surveys are often influenced by the fact that most of the authors test only cats brought into a veterinary ambulance.

The risk of disease occurrence based on the study of Holičková[32] is nearly five times higher in cats with clinical symptoms of the disease in comparison with cats without clinical symptoms.

C. felis causes a chronic infection of the organism, whereupon the organism excretes the pathogen for many months. Helps et al[5] describes the impact of the environment in which the cats live, focusing on the concentration of animals and animal hygiene conditions at breeding farms and animal shelters. The highest proportion of co-infected cats was accounted for the cats living in shelters or deposit devices (private amateur objects without inspection) or animal shelters. The shelters or deposit devices will easily become the source of the infection of the stray cats and domestic cats under infringement of the rules of animal hygiene, isolation and impact treatment of all animals in the shelters or deposit devices. A more recent study by Holičková et al[30] has confirmed that stray and shelter cats may be important sources of feline chlamydiosis and may present a risk for transmission to humans. Despite several studies, the risk factors were not precisely set for the occurrence of this infection.

C. felis may induce conjunctivitis in people who are in close contact with infected cats;[3] however, the risk is very low, as demonstrated by the higher seroconversion in cats than in cat owners.[2] *Chlamydiaceae* are not the only etiological agents of certain infectious diseases, but they also display a synergic impact on other micro-organisms, such as viruses and bacteria.[39] Thereby the virulence of other microorganisms increases.[40] Moreover, it has been shown that a secondary infection can accelerate the clinical course of the infection of FIV, prolonging the duration of clinical symptoms, which leads to chronic symptoms of disease.[8] Bearing in mind that FIV reduces the efficiency of immune responses, the result of co-infection may be a higher susceptibility for other pathogens,[41] such as FeLV, and leading to a progressive disease with permanent viraemia.[39,42] The oral and conjunctival

microflora likely play an important role in prevention of opportune infections, although the microbes may also be a source of potential pathogens. In case of immunosuppression induced by feline retroviruses, the overgrowth of microflora can occur.[43]

Weese et al[43] analyzed a collection of oral and conjunctival swabs from the cats with and without FIV infection. They tested 19 FIV positive and 13 FIV negative cats. *Chlamydiaceae* were confirmed in both the FIV-negative and the FIV-positive cats to the same extent. The confirmed high prevalence of Chlamydia infections in FIV-positive cats indicates the significance of co-infections, given the more severe progression of the disease, as corroborated by a study from Romania.[38]

Analysis of FIV and FeLV Diseases

The prevalence of FeLV and FIV may vary based on different subgroups or the geographical origins of cats and its impact on the occurrence of clinical symptoms of the disease. In our research, we also focused on the impact on the occurrence of clinical symptoms of disease.

The most frequent clinical symptoms of the disease diagnosed with FIV positive cats were anorexia (14/26), gingivitis and stomatitis (12/26), pyrexia (10/26), and diarrhea (9/26). Based on these results, our study identified pyrexia, anorexia, cachexia, gingivitis and stomatitis as accompanying phenomena of FIV disease.

It can be stated that anorexia, diarrhea, cachexia, gingivitis with stomatitis, and lymphadenopathy belong to the accompanying phenomena of FeLV disease.

Our calculation of the relative risk showed that pyrexia, apathy, infection of upper respiratory tract, conjunctivitis, blepharospasm, ocular discharge and factors such as sex, area of capture (city, suburban area and rural area) are statistically insignificant for FeLV disease.

On the other hand, we proved that diarrhea, lymphadenopathy, anorexia, gingivitis and stomatitis and cachexia are statistically significant symptoms for FeLV disease. These results suggest that identifying the symptoms we evaluated may contribute to estimating the prevalence of FeLV in the cat population.

Co-Infections with FeLV/Chlamydia Spp

Co-infections with FeLV/*Chlamydia* spp. were not detected in our study.

For reference, in a study from Slovenia, the prevalence of FeLV/*C. felis* co-infection was 6.67%,[34] in 3.33% of cats, the co-infection FeLV/*C. felis* was not accompanied by clinical signs.

Analysis of Co-Infection with FIV/Chlamydia Spp

It can be concluded that co-infection leads to exacerbation of symptoms and thus to an aggravation of clinical symptoms of infectious diseases[44] in immunosuppressed cats. It can be said that this is the terminal stage of FIV infection, when the secondary infection leads to the death of the individual. Euthanasia is recommended in such cases.

In our study, we confirmed that the risk of *Chlamydia* spp. infection is 2.13 times higher in FIV positive cats and the risk of FIV infection is 2.10 times higher in *Chlamydia* spp. positive cats. Relative risks calculated for these cases were significant. In comparison, in Slovenia, the prevalence of co-infection with FIV/*Chlamydia* spp. was reported to represent 10.00% of positive animals,[34] in 3.33% cases the co-infection was not accompanied by any clinical signs.

Analysis of Co-Infection with FIV/FeLV/Chlamydia Spp

Co-infection of FIV/FeLV/*Chlamydia* spp. was confirmed in 2 free-roaming cats out of 168 cats. The prevalence was 1.19%. In 36 free-roaming cats showing clinical symptoms, two cats were FIV/FeLV/*Chlamydia* spp. positive (prevalence 5.56%). Both cats were older than one year, female, indoor-outdoor. The cats were brought to and examined in a veterinary ambulance in the terminal stage of the disease. Euthanasia of cats was subsequently performed.

It must be taken into account that the number of cat infected by FIV/FeLV/*Chlamydia* spp. is too small for evaluation and therefore is insignificant.

Sykes[45] observed that 8% of all cats in their study were co-infected by *C. felis* with feline calicivirus (FCV) or feline herpesvirus type 1 (FHV-1),[31] 64% of the cats tested positive for *C. felis*, DNA of FHV-1 infection was detected at the

same time.[45] A correlation between FIV, FCV, FHV-1 and FeLV was found in a more recent study as well.[46] Cats with chronic FHV-1 infection are more prone to co-infections of FeLV and FIV. Our study confirmed that the risk of *Chlamydia* spp. infection in FIV/FeLV positive cats and the risk of FIV/FeLV infection in *Chlamydia* spp. positive cats were statistically insignificant in calculations of relative risks.

Analysis of Co-Infection with FIV/FeLV

Co-infection of FIV/FeLV was confirmed in 5 free-roaming cats out of 168. The prevalence was 2.97%. An asymptomatic course of the disease was observed in 1 cat (0.60%).

Studies in other countries reported the following prevalence of FIV/FeLV co-infection: in Slovenia, the prevalence of FIV/FeLV was 3.33%,[34] the authors of the study reported that cats in which co-infection with FeLV/*C*. felis were without any clinical signs. An extensive study in Malaysia detected a 2.6% prevalence of FIV/FeLV co-infection.[47] In Brazil, the co-infection with FIV/FeLV was detected in 1 of 142 (0.7%) sampled cats.[48] A recent study from Thailand[49] found multiple cats living in one place to be a risk factor in developing a FIV/FeLV infection in cats, with the prevalence of co-infection 2.7%.

The clinical symptoms often associated with FIV/FeLV diseases are cutaneous abscesses,[50] gingivitis and stomatitis,[51,52] lymphoma and anemia.[39,41,52,53]

The results of our study confirmed that the risk of FIV infection in FeLV positive cats was statistically insignificant in calculations of relative risks. Our results suggest that identifying the symptoms we evaluated may contribute to estimating the prevalence of FeLV in the cat population.

In case of free-roaming cats, vaccination against FeLV and chlamydiosis represents the most important preventive measure. In cases of co-infections with FIV/*Chlamydia* spp. and FIV/FeLV/*Chlamydia* spp., in which the signs of the diseases are already manifest, euthanasia should be considered. We do not recommend treatment or vaccination of these cats due to the resulting deepening of their immune suppression. Each cat should be tested before vaccination.

Conclusion

The prevalence of FIV, FeLV and *Chlamydia* spp. resulting from our research indicates that the occurrence of these pathogens in the populations of free-roaming cats is endemic. The results of our tests suggest that the number of animals with a diagnosed infectious disease is higher in locations with higher population density of cats. Our study found more than one pathogen to be correlated with more severe disease symptomatology.

Ethical Approval

The Ethics Committee of the University of Veterinary Medicine and Pharmacy referenced from EKVP/2023-02 granted authoritative approval for the handling of the animals, sample collection and use of the samples in our study. Informed consent was obtained from the participants or animal owners without harming the welfare of the animals.

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Disclosure

The authors report no conflicts of interest in this work. Animal rights statement: The study was performed in accordance with the institutional guidelines for animal welfare issued by the Ethical Committee of the University of Veterinary Medicine and Pharmacy in Košice. Informed consent was obtained from all the animals' owners.

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