Impact of way of life and environment on the prevalence of *Chlamydia felis* in cats as potentional sources of infection for humans

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Abstract

Introduction and objectives. *Chlamydia (C.) felis* can cause infection which may be associated with conjunctivitis and/or respiratory tract disease, particularly in kittens, but could also be the cause of the disease in adult cats. Infection is more common in multi-cat environments. The zoonotic potential of *C. felis* appears low, but exposure to this microorganism is possible by handling the affected cats, by contact with their aerosol, and also via fomites.
Materials and method. In the study, 140 cats of various breeds from Košice region in Slovakia were studied. Conjunctival samples were obtained from 71 clinically healthy cats (50.7%) and 69 cats with clinical signs of conjunctivitis and upper respiratory tract impairment (49.3%). Cats were divided into 4 groups according to breed and type of environment in which they lived. In the 1st group were cats kept inside only (n=33), in the 2nd group, free-roaming cats (n=50), the 3rd group comprised stray cats, taken from the streets (n=28), and the 4th group included cats kept in shelters or deposit devices (n=29). Molecular method PCR and DNA sequencing was used as the diagnostic method.
Results. Overall positivity was 17.1%. Of the 24 positive cats, the highest positivity was detected in the population of stray cats (35.7%) and shelter cats (31%). In the group of free-roaming cats, 10% had positivity. No positive animals were detected in the group of cats kept inside only. It was also found that the risk of *C. felis* in cats with clinical signs of disease was more than 7-fold higher than in cats without clinical signs of conjunctivitis and respiratory tract.
Conclusion. The obtained results show that cats, especially stray and shelter cats, can be important sources of feline chlamydiosis, and due to their close contact with people they can present a risk for transmission.

Key words
Chlamydia felis, cat, prevalence, zoonosis, PCR, public health

INTRODUCTION

The interaction between human and animal health is not a new phenomenon. However, the scope, scale, and worldwide impact of zoonoses we are facing today have no historical precedent. Of the 1,415 identified infectious pathogens for humans 868 (61%) have a zoonotic character [1], and these diseases have an increasing trend, not only in developing but also in developed countries. The ecological changes caused due to the destruction of the natural environment increase the exposure of animals and humans to the vectors and reservoirs of unknown pathogenic microorganisms. In addition to this, weak public health systems, other natural disasters, absence of better surveillance and controlling systems against the disease and unhygienic living conditions are the main factors for the emergence and re-emergence of the bacterial zoonotic diseases in many countries worldwide [2]. The rapid detection and response to diagnostic diseases is crucial, because on many occasions there is an unobserved spreading and infiltration of diseases to new territorial units,

especially in the case of emerging, respectively, re-emerging diseases.

One of the most common microorganisms in nature which have a zoonotic character are chlamydiae from the family *Chlamydiaceae*. *Chlamydiaceae* has only a single genus, *Chlamydia* that comprises 12 species [3, 4], including *Chlamydia felis*. *Chlamydia felis* is an obligate intracellular bacterium that seems to have a predilection for conjunctival epithelial cells, and is the most common cause of feline chlamydiosis and different diseases in humans.

Feline chlamydiosis is the most common cause of pneumonia and acute or chronic conjunctivitis, particularly in kittens [5, 6, 7], but could also be the cause of the disease in adult cats [8]. Clinical signs of infection with *C. felis* in cats are sneezing, transient fever, inappetence, weight lost, nasal discharge, vaginal discharge and lethargy. Chlamydial disease may be complicated by coinfection with other microorganisms. Feline chlamydiosis is more common in multi-cat environments, particularly breeding catteries where animals share a common space [9].

C. felis spreads through direct contact between cats, and cats and humans. Transmission between cats is due to direct contact with infectious eye secretions. This pathogen is highly unstable outside the host and remains infectious for a short time in the environment.

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The zoonotic potential of these bacteria seems to be low; however, *C. felis* may be exposed to the manipulation of infected cats. In particular, cat owners and employees working with cats (veterinarians, breeders'staff) are at increased risk, especially where poor hygiene conditions are present. The risk of zoonotic transmission is likely to be greater in immunocompromised individuals [10, 11, 12, 13, 14]. In general, acute conjunctivitis is a frequent human condition, with an estimated six million cases per year in the USA, and *Chlamydiae* being the cause in 1.8–5.6% of all cases [15]. However, only few human cases of *C. felis* conjunctivitis have been reported, despite its high incidence in cats and the large number of pet cats in society. Actual prevalence may be much higher because human infection is predominantly asymptomatic [16].

OBJECTIVE

Because the control of incidence of chlamydial infections between animals and humans is the key to a better understanding of the relationship between chlamydiae and their hosts, the aim of this study was to investigate the incidence of *C. felis* in cats, according to breeding and the type of environment in which they live, and to compare the incidence in cats with and without clinical signs of infection, and assess the zoonotic potential.

MATERIALS AND METHOD

Samples were collected from cats examined at the Department of Small Animals at the University of Veterinary Medicine and Pharmacy in Kosice, Slovakia, and various deposit centres (shelters) in Kosice, Slovakia. 140 cats of various breeds, aged from 1 month to 10 years were examined. Most of the examined cats were European shorthair cat breeds (ESH); lesser represented breeds were Maine Coon, British blue and Sphinx. Based on the type of environment, the animals were divided into 4 groups for examination:
1) cats kept inside human habitations, without possible contact with other cats and other animals. A requirement for inclusion in this group was also internal breeding. Total: 33 cats.
2) Free-roaming domestic cats, able to move around freely, and thus come into contact with other cats, respectively, animals. Total: 50 cats.
3) Stray cats taken from the streets and brought to the clinic for examination. Total: 28 cats.
4) Cats housed in shelters (so-called deposit facilities), characterized by a high concentration of animals. Total: 29 cats.

In each of the 4 groups, the cats were also divided on the basis of clinical signs of chlamydial infection. A total of 140 cats, 69 had clinical signs and the other 71 were without any pathological finding. None of the examined cats were vaccinated against chlamydia infection.

After administration of local anesthetic into the eye, samples were taken with a sterile cotton swab rolled deep into conjunctival sac to capture the biggest number of epithelial cells of the conjunctiva. Immediately after sampling from each eye, the cotton swab was macerated in tubes filled with 300μl saline solution for 60 seconds. Samples thus obtained were stored at -80 °C until testing.

Molecular analysis. For isolation of the DNA, samples resulting from mixing 150μl samples from both eyes were used. DNA isolation was carried out according to the manufacturer's instructions using a commercial diagnostic kit DNA-Sorb/AM (Nucleic Acid Extraction Kit, AmpliSens, Federal State Institution of Science, Moscow, Russia). In cases where the amplification was not been carried out immediately, the samples were stored at -20 °C until their next use. Positive specimens for *Chlamydiaceae spp.* were subsequently amplified using a set of primers U23Fa (5'-GAT GCC TTG GCA TTG ATA GGC GAT GAA GGA-3') and 23 SIGR (5'-TGG CTC ATC ATG CAA AAG GCA-3') that amplify domain I of 23S rRNA gene. The reaction mixture for one reaction was prepared by mixing 0.2 μl 10 pmol of primer U23Fa, 0.2 μl 10 pmol of primer 23 SIGR, 9.6 μl of purified water, 4 μl of Taq polymerase (HOT FIREPol Blend Master Mix, Solis BioDyne, Estonia), and 6 μl of template (isolated DNA). Electrophoresis was carried out for 20 minutes at 300V. PCR products were analysed by electrophoresis of 10 μl of each 20 μl reaction mixture on a 1.5% agarose gel stained using GoldView stain (SBS Genetech, China) and visualized fragments were compared with the positive control and the 100 bp DNA ladder.

Positive products *Chlamydiaceae spp.* were compared against the positive control for the length of 600bp under UV light at a wavelength of 254nm. The identity of the obtained sequences was examined by a BLAST search [17].

DNA sequence alignments and phylogenetic analysis were conducted using software MEGA5. Phylogenetic trees were created using alignments performed with the BioEditSequence Aligment Editor as a distance method and NJ (neighbour joining) as the tree construction method. All ambiguous positions were removed from each sequence pair. The reliability of branches in trees was assessed using bootstrap analysis with 1,000 pseudoreplicates, with values above 70% being noted.

Analysis of sequences for constructing the phylogenetic tree also included 9 reference strains, the partial sequence of the 23S rRNA gene of different chlamydial species from the Gene Bank database, *C. felis* FP Cello (U68458), *C. felis* VR 120 (U68457), *C. pneumoniae* (U68424), *C. suis* (U68420), *C. muridarum* (U68436), *C. pecorum* (U68439), *C. avium* (NR_121988), *C. abortus* (U68444), *C. caviae* (NR_076195), and *Pirellula marina* (AF 245367) was used as the outgroup.

Statistical analysis. Basic descriptive statistics were used for the analysis of the obtained results. To compare the incidence of chlamydial infections between the groups, a calculation of the relative risk (RR) and their 95 percent confidence intervals (95% CI) was performed and estimated for the occurrence of *C. felis* infection. All tests were considered significant at $p < 0.05$.

RESULTS

Determination of the prevalence of *C. felis* infection in the population of cats in Slovakia and its comparison with stray, domestic cats and cats kept in shelters. Of the 140 cats examined by PCR, *Chlamydia spp.* was detected in

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24 (17.1%). After amplification and electrophoretic analysis, positive samples were sent for sequencing. By analyzing the sequences of the 23S rRNA gene to construct the phylogenetic tree using 9 reference strains of different species of chlamydia, in all positive cases the species *C. felis* was confirmed (Fig. 1).

Comparison of occurrence of C. felis infection in cats depending on clinical signs and farming method. When comparing the occurrence of *C. felis* in cats with conjunctivitis and/or clinical signs of respiratory tract disease, compared with cats without clinical signs, 30.4% positive for cats with clinical signs were confirmed versus 4.2% positive for cats without clinical signs. When calculating the relative risk between these groups, it was found that the risk of *C. felis* in cats with clinical signs of disease was more than 7-fold higher (P = 0.0095) than in cats without clinical signs of conjunctivitis and respiratory tract infection (Tab. 1).

The most common clinical sign of chlamydial infection was conjunctivitis with prevalence of purulent discharge, chemosis, hyperaemia, blepharospasm and mucopurulent discharge.

When comparing the occurrence of chlamydia infection, according by the farming method, it was found that stray cats and cats from shelters and deposits (35.7% and 31%, respectively) were infected most frequently. In the group of free-roaming cats, from the 50 examined, 5 were positive (positivity 10%). None of the cats from the group kept strictly within human habitations were detected chlamydial infection (Tab. 2).

By comparing the relative risk, it was found that compared to cats strictly kept inside, free-roaming cats had more than a 7-fold risk of chlamydial infection, cats from shelters and deposits had this risk of more than 21-fold, and stray cats almost 25-fold (Tab. 2).

Table 1. Occurrence of *C. felis* depending on occurrence of clinical signs

Cats	No. of examinations		No. of positive case		Relative risk (95% CI)
	N	%	N	%	
with clinical signs	69	49.3	21	30.4	7.,2 (2.25 – 23.06)
whithout clinical signs	71	50.7	3	4.2	1*
Σ	140	100	24	17.1	

*1 – reference group

Table 2. Prevalence of *C. felis* in cats by groups

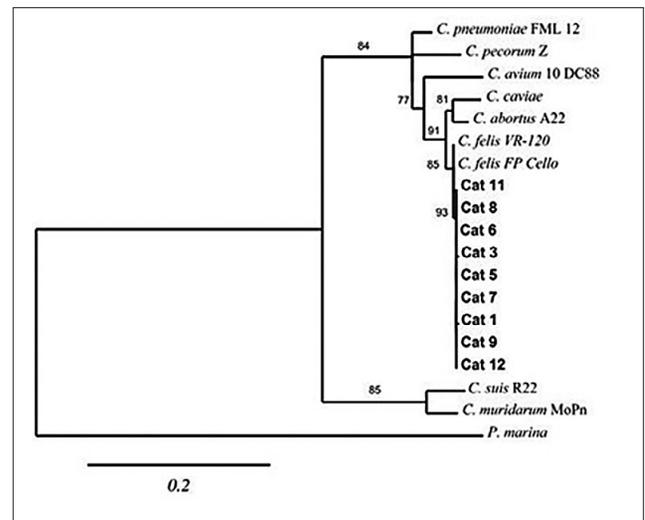| Group	No. of examinations		No. of positive case		Relative risk (95% CI)
	N	%	N	%	
cats strictly kept inside	33	23.6	0	0	1*
free-roaming cats	50	35.7	5	10	7.3 (0.41 – 128.35)
stray cats	28	20	10	35,7	24.6 (1.50 – 402.25)
cats from shelters and deposits	29	20.7	9	31	21.5 (1.30 – 354.52)
Σ	140	100	24	17,4	

*1 = reference group

Figure 1. Phylogenetic tree of C. felis isolates from the presented study (cat) and selected accessions of chlamydial species from GenBank, based on 23S rRNA gene fragment sequences

DISCUSSION AND CONCLUSIONS

With the growing interest in the breeding and adoption of cats from shelters in Slovakia, the question arises about the prevalence of certain infectious diseases of cats. Conjunctivitis and respiratory diseases are frequently occurring clinical symptoms in young stray cats [18]. One of the major pathogens in cats is *Chlamydia felis*, which is a bacterial agent of upper respiratory tract infection and conjunctivitis.

C. felis is endemically spread between domestic cats worldwide, and its prevalence has been published in several studies [7, 19, 20, 21, 22, 23]. A 17.1% prevalence of *C. felis* infection was found Slovakia, diagnosed by the PCR method. Of the total number of healthy cats (n = 71), *C. felis* infection only in 3 cases, representing a 4.2% prevalence of chlamydiosis in healthy cats. This result correlates with studies published in other countries. Prevalence of *C. felis* in clinicaly healthy cats in Italy is 3.3% [24] and in the USA – 0% [25]. In clinically healthy animals from several European pedigree catteries or shelters, the prevalence of *C. felis* was 3% [21].

In the presented study, the prevalence of *C. felis* infection in the population of cats showing clinical signs was 30.4%. The relative risk of the disease is 7 times higher (P = 0.0095) in cats with clinical signs of the disease than in cats without clinical signs.

Feline chlamydiosis is one of the most common disease in cats, but there is very little information regarding the prevalence of the disease in Slovakia. Prevalence of *C. felis* in Slovakia confirmed by IFA has been published previously [22]. In the current study, the total prevalence of *C. felis* in cats with clinical signs of conjunctivitis was 45.16%, with the highest proportion of positive cats comimg from shelters (65.8%). Since there is a lack of studies focused on screening for capturing the most common pathogens of conjunctiva and upper respiratory tract in Slovakia, proportion of *C. felis* pathogen in total infections in these inseparable systems in cats remains unclear.

In other studies, the prevalence of *C. felis* in cats with upper respiratory tract disease determined by culture is in the range of 23 – 31% [26, 27]. Studies using PCR of

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samples from conjunctiva led to the specification of the prevalence of 14.3% (66/462) in cats with upper respiratory tract disease (URTD) [28]; 17.7% (20/113) in cats with symptoms of conjunctivitis [29]; 20% (14/70) in cats also with conjunctivitis [20], and 11.5% (26/226) in Swiss cats with eye symptoms [30]. Differences in prevalence may be due to different diagnostic methods. It seems that the significance of *C. felis* as a causative agent of URTD and conjunctiva differ among countries. While in the USA this agent is considered less important than feline herpesvirus 1 (FHV1) [31], *C. felis is* the most common pathogen causing conjunctivitis and URTD in Japan [32]. An explanation may be the difference in the prevalences in these countries.

In the current study, almost all the infected cats had clinical symptoms of conjunctivitis. The most common clinical symptom of conjunctivitis was purulent discharge from the eyes, and affected 50% of the infected cats. Some literary sources report an increased incidence of chemosis and less severe mucosal discharge [8, 18]. However, not all affected cats show symptoms of conjunctivitis, and therefore the absence of clinical signs does not indicate the absence of infection. Sparkes et al. (1999) [33] reported that the clinical symptoms may subside in untreated cats within 3 months, but the excretion of *C. felis* can continue for at least 8 months. The prevalence of *C. felis* in asymptomatic cats is low. In studies using PCR was <5% [20, 24, 28, 30]. Persistent excretion of chlamydia after subsidence of clinical signs may be an explanation for the positivity in some asymptomatic cats [30].

Several risk factors for this infection have been described, especially the impact of the environment in which cats live, the concentration of animals in the room and zoohygienic conditions in breeding farms and animal shelters. Previous studies show that up to 95% of infected cats came from shelters with poor zoohygienic conditions [21]. Also, an important risk factor in the transmission of infection is the presence of other animals. Studies by Binns et al. (2000) [34] and Dawson et al. (2000) [35] have shown that a significant proportion of the incidence of upper respiratory tract disease may be played by the presence of dogs. A potential source of infection of cats are also birds and rodents [36, 37]. The disadvantage of deposit centres is that they often have other kinds of animals, such as dogs and exotic birds.

In the current study, 0% prevalence was found in household cats kept strictly indoors. In the population of free-roaming domestic cats, which have the possibility of contact with other cats and animals, the prevalence was 10%, which is comparable to the 11.5% prevalence in Australia [7]. Gruffyd-Jones et al. [9] found in their study the highest prevalence of chlamydiosis in breeding farms and shelters. The prevalence in shelters and stray cats, according to the current study, was similar and represents 31% and 35.7%, respectively. In another study by Helps et al. (2005) [21] who studied 1,748 cats from 218 shelters in different European countries, found that *C. felis* was at the level of 10% in affected cats and 3% in healthy cats. Similarly, other epidemiological studies found differences in the incidence of *C. felis* infection in cats, depending on the farming method. In a study that analyzed a shelter with a high density of cats of unknown origin, and had a history of upper respiratory tract infection and acute and chronic conjunctivitis, a high incidence (58.06%) of *C. felis* in the cats was found in comparison with previous studies. These data suggest that a high density of animals may be associated with the spread of the agents. All cats in this the study had

clinical signs of upper respiratory tract infection, and most cats positive for *C. felis* had a mucopurulent discharge from the eyes or nasal secretion [38]. These differences are likely to be attributed to the lifestyle and animal welfare.

The most important reservoirs of chlamydia, mainly *C. psittaci*, are feral pigeons which are widespread in several European cities and towns. The prevalence in these birds ranged from 1.6% – 95.6% [39, 40, 41, 42]. Stray cats have more opportunities to interact with infected birds or other animals, and suffer from inadequate nutrition and possibly weakened immune systems that can contribute to increased exposure to infectious pathogens. This may explain the higher prevalence of *C. felis* in stray cats than in cats kept strictly indoors.

This also raises the issue non-existent shelters for cats in Slovakia. At present, the problem of catching and placing the cats is solved through the so-called deposit centres. The owners of these „shelters" are private individuals who are trying to co-operate with a non-profit organization to help tackle the issue of adopting cats from the streets, thereby reducing the number of cats and their total population. Cats are held at these deposit centres/shelters until owners are found for them which, in some cases, may take several months or even years. The arrival of other new animals in such an environment causes the mutual exchange of various types of infections, including chlamydia, and becomes a reservoir of diseases for the healthy cats. The starting point of a poorly-funded deposit centre and a lack of awareness by the temporary owners of the cats, is the death rate, often affecting up seversal cats among a high concentration of animals. By complying with the principles of zoohygiene, quarantine, therapy and vaccination of animals in shelters and breeding colonies, the incidence of the disease should be minimized. Based on the identified risk groups, the vaccination of the cats is recommend. Live attenuated vaccines or inactivated vaccines are available, but their use should be restricted to animals exclusively at risk.

Zoonotic risk of *C. felis* is known and its transmission to humans has been documented in several studies [10, 14, 43, 44]. In humans, *C. felis* may cause conjunctivitis, and/or respiratory tract diseases, [10], pneumonia [11], hepatosplenomegaly, glomerulonephritis and endocarditis, especially in those who live in close contact with cats [12]. They, may also cause abortion, neonatal mortality and infertility [13]. However, as a study of seroprevalence between cats and their owners has shown, the risk of infection is very low [6].

The obtained results show a high proportion of *C. felis* in the etiology of infections of the conjunctiva and upper respiratory tract, and an endemic presence of this pathogen in the population of stray and shelter cats. The probability of the infection in cats that have no possibility of contact with other cats and animals is very low, and the same is the case of animals without clinical signs of disease. The risk of the disease in cats with clinical signs of infection is 7 times higher than in cats with no clinical symptoms, but it can also be found in clinicaly healthy cats.

Cats could play an important role in the transmission of chlamydiosis to humans. By understanding and following the principles of hygiene, quarantine, treatment and vaccination of animals in shelters and breeding colonies, the occurrence of the infection could be decreased.

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