Original Article

Molecular Detection of *Chlamydia felis* in Cats in Ahvaz, Iran

Barimani [1], M., Mosallanejad [1], *, B., Ghorbanpoor Najafabadi [2], M., Esmaeilzadeh [2], S.

1. *Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran*
2. *Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran*

ABSTRACT

Chlamydiae are obligate generally Gram-negative intracellular parasites with bacterial characteristics, including a cell wall, DNA, and RNA. They have a worldwide distribution in different animal species. *Chlamydia felis* (*C. felis*) is an important agent with zoonotic susceptibility often isolated from cats with chronic conjunctivitis. The aim of the present survey aimed to determine the molecular occurrence of *C. felis* in cats in Ahvaz, Iran. In this regard, a total of 152 cats (126 households and 26 feral) were included in the current study. After recording their history information, two swabs were taken from the oropharyngeal cavity and eye conjunctiva of the investigated cats. The extraction of DNA was followed by PCR targeting the pmp gene of *C. Felis*. In the next step, the positive samples were sequenced based on the Gene Bank. Out of 152 samples, 35 (23.03%) were positive using polymerase chain reaction technique (95% CI: 16.30-29.70). Regarding infection with Chlamydiosis, the obtained results showed a significant difference between cats suffering from ocular or respiratory diseases (44.64%; 25 out of 56) and the healthy ones (10.42%; 10 out of 96; P=0.01). The prevalence of infection was significantly higher in cats younger than 1 year (34.12%; 29 out of 85), compared to those older than 1 year (8.96%; 6 out of 67; P=0.02). No significant difference was noted in terms of gender (25.45% in males and 21.65% in females), breed (23.81% in DSH and 19.23% in Persian), and lifestyle (22.22% companions [28 out of 126] and 26.92% ferals [7 out of 26]; P>0.05). It can be concluded that a significant number of cats are infected with *C. felis* in Ahvaz. The use of molecular tests, such as PCR, has revolutionized the diagnosis of chlamydial infections.
Keywords: *Chlamydia felis*, Chlamydiosis, PCR, Ahvaz, Cat

Détection Moléculaire de *Chlamydia felis* chez des Chats à Ahvaz, en Iran
Résumé: Les *Chlamydia* sont généralement des parasites intracellulaires à Gram négatif avec des caractéristiques bactériennes, notamment une paroi cellulaire, de l'ADN et de l'ARN. Ils ont une distribution mondiale et affectent différentes espèces animales. *Chlamydia felis* (*C. Felis*) est un agent important susceptible de causer une zoonose, souvent isolé chez les chats souffrant de conjonctivite chronique. Cette étude avait pour objectif de déterminer l'occurrence moléculaire de *C. felis* chez des chats d'Ahvaz, en Iran. A cette fin, un total de 152 chats (126 d'animaux domestiques et 26 chats errants) a été includans notreétude. Après avoir enregistré leurs renseignements médicaux, deux écouvillons ont été prélevés à partir de la cavité oropharyngée et de la conjonctive des chats soumis à l'enquête. L'extraction de l'ADN a été suivie d'une PCR visant le gène pmp de *C. Felis*. ensuite, les échantillons positifs ont été séquencés sur la base des données disponibles dans la banque de gènes. Sur 152 échantillons, 35 (23,03%) étaient positifs en utilisant la technique de réaction en

chaîne de la polymérase (IC à 95%: 16h30 à 29,70). En ce qui concerne les taux de Chlamydiose, nos résultats ont montré une différence significative entre les chats atteints de maladies oculaires ou respiratoires (44,64%; 25 sur 56) et les chats en bonne santé (10,42%; 10 sur 96; p = 0,01). La prévalence de l'infection était significativement plus élevée chez les chats de moins d'un an (34,12%; 29 sur 85) comparés à ceux âgés de plus d'un an (8,96%; 6 sur 67; p = 0,02). Aucune différence significative n'a été notée selon le esexe (25,45% chez les hommes et 21,65% chez les femmes), la race (23,81% chez DSH et de 19,23% en persan) et le style de vie (22,22% chez les chats domestiques [28 sur 126] et 26,92% chez les chats errants) [7 sur 26]; P> 0,05). On peut en conclure qu'un nombre important de chats sont infectés par *C. Felis* à Ahvaz. L'utilisation de tests moléculaires, tels que la PCR, a révolutionné le diagnostic des infections à *Chlamydia*.

Mots-clés: *Chlamydia felis*, Chlamydiosis, PCR, Ahvaz, Chat

INTRODUCTION

Chlamydiae are obligate generally Gram-negative intracellular parasites with bacterial characteristics, including a cell wall, DNA, and RNA. Recent years have seen expansion in the family Chlamydiaceae. Today, this family includes 13 species belonging to the genus chlamydia, namely *C. trachomatis*, *C. pneumoniae*, *C. abortus, C. Caviae, C. felis C. muridarum, C. pecorum, C. Psittaci*, *C. suis, C. avium*, *C. gallinacean*, *C. serpentis*, and *C. poikilotheymis* (Bommana et al., 2019). The *C. felis* was first isolated in the United States in 1942 from cats with Upper Respiratory Tract Disease (URTD). Several studies have been reported that *C. felis*, feline calicivirus, and feline herpesvirus type-1 are the major causative agents in URTD (Bannasch and Foley, 2005; Kang and Park, 2008; Hartmann et al., 2010). Cats infected with *C. felis* develop clinical signs, including fever, ocular discharge, and sneezing. The predominant feature is the inflammation of the conjunctiva or nictitating membrane. Ocular signs in cats range from chemosis, hyperemia, blepharospasm, and mucopurulent to serous ocular discharge. Conjunctivitis may persist for several months in cats. Infections with *C. felis* often become chronic, insidious, and transmitted through close direct or aerosol contact. In this regard, although the cats may be asymptomatic carriers of the infection, they harbor the organism in the conjunctiva for 2 months or longer. Organisms have been isolated from the conjunctiva for up to 215 days after experimental infection with no breed or gender predilection. Previous studies have shown that the discrepancies on the prevalence of *C. felis* infection in the cats are due to the employed techniques, sample size, geographic locations, and species of cats (domestic or feral). The isolation rate of *C. felis* is up to 30% in household cats with conjunctivitis (Sykes and Greene, 2012). Many stray cats live in the urban areas of Iran, which are in contact with other animals. This issue increases the risk of health among not only animals but also humans. The obtained results of the surveys in Iran were indicative of a relatively high prevalence of *C. felis* in cats. For example, the findings of a study conducted in Tehran and Isfahan revealed that 40 cats (17.85%) out of 224 cases were infected with *C. felis* (Momtaz et al., 2014). In another study carried out in Tehran, the prevalence of this infection was reported at 20% in cats (Maazi et al., 2016). There are many different methods for the diagnosis of Chlamydiosis in animals, such as cell culture (cultivation), cytology (conjunctival swabs), genetic detection, direct fluorescent antibody test, indirect fluorescent antibody test, enzyme-linked immunosorbent assay (ELISA), and polymerase chain reaction (PCR). Molecular assays (e.g., PCR) are becoming increasingly available for the diagnosis of Chlamydia infections in cats. These methods are rapid and sensitive, and they allow the speciation of isolates from cultures or

infected tissues. The PCR method followed by restriction digestion or high resolution melting curve analysis can be used to differentiate the species of *Chlamydiaceae*. Furthermore, this method does not need a viable organism for testing (Helps et al., 2003; Sykes and Greene, 2012). To the best of our knowledge, there is no study addressing Chlamydiosis in this area; therefore, the purpose of the present study was the molecular detection of *C. felis* in companion and feral cats of Ahvaz, Iran.

MATERIAL AND METHODS

Sample population. This cross-sectional survey was conducted in Ahvaz in south-western Iran from December 2016 to October 2017. A total of 152 cats (97 males and 55 females) were included in the present survey. The household cats (126 cases) were referred to the Veterinary Teaching Hospital of Shahid Chamran University of Ahvaz, Iran, for vaccination, health care, and diagnosis of different diseases, including respiratory and ocular diseases, as requested by the owners. The feral cats (26 cases) were taken from different areas of Ahvaz. All parameters including age, gender, breed, clinical signs, lifestyle, and vaccination history were recorded. The breed distribution indicated that 82.89% (n=126) and 17.11% (n=26) of the studied cats were Domestic short hair (DSH) and Persian. The studied cats were categorized into two groups based on age (younger and older than 1 year). The age the investigated cats ranged from 2 months to 9 years (median 1.4 years). Local anesthetic (tetracaine 1%) was administered in the cats' eyes to facilitate sampling. In some cases, sedative drugs (e.g., ketamine with dosage 15 mg/kg and acepromazine 0.15 mg/kg) were injected as intramuscular for sedative effects, and then two swabs of oropharyngeal cavity and eye conjunctiva were taken from the investigated cats. Those cats treated with antibiotics, such as oxytetracycline, at the time of referral were excluded from the study. Eye samples were taken using suitable sterile swabs over the ventral conjunctiva. Both conjunctival and oropharyngeal swabs of each cat were placed in a single 15ml tube containing 2.5 ml of sterile sucrose phosphate transport (SPG) buffer (Johnson, 1984) and considered as a single sample stored at -70oC until testing.

DNA extraction. Swabs in each tube are vortexed in SPG buffer and after the centrifugation at 5000 rpm for 5 min, the DNAs of precipitates were extracted using a G-spin total kit (iNtRON, South Korea), according to the manufacturer's instructions. The extracted DNAs were maintained at -20 °C until PCR testing.

PCR. Specific primers were designed for the amplification of *C. felis* pmp1 specific gen (Gen Bank Accession No. EF092092), which encoded chlamydial polymorphic membrane protein (Cantekin et al., 2014). Specific primers were also used for the mitochondrial cytb gene of Felis catus (Gen Bank accession No. AB194813) as internal amplification control (Cantekin et al., 2014). The properties of the employed primers (names, target gene, sequence, and references) are shown in Table 1. Doubleplex PCR was performed in a total volume of 25 µl, including 12.5 µl of master mix 2x (Amplicon, Denmark, consisting: 2x PCR buffer, 200 μM of the dNTPs, 2 mM Mg Cl2 and 0.5 U of Taq DNA polymerase) with 4 µl of primer mix (20 mM of each 4 primers), 5 µl of extracted DNA (template), and 3.5 µl of PCR grade distilled water. The PCR reactions were performed in Eppendorf thermo-cycler (Eppendorf, Mastercycler® 5330, Germany). Thermal conditions were an initial denaturation for 10 min at 94 °C, 35 cycles of 30 sec at 94 °C, 30 sec at an annealing temperature of 58 °C, synthesis at 72 °C for 30 sec, and final elongation at 72 °C for 10 min. The PCR products were exposed to electrophoresis for 1 h at 70V in 1.5% agarose gel containing safe stain (Sinaclon, Iran). The results were visualized and photographed under ultraviolet illumination (Uvitec, England). The DNA from *C. felis* vaccine (Fel-O-Guarde plus 4, Boehringer Ingleheim, USA) and PCR grade distilled water were used in each reaction as positive and negative controls, respectively.

Statistical analysis. The studied cats were grouped by age, gender, breed, clinical signs (ocular or respiratory) and lifestyle (companion or feral) to determine whether these factors were associated with *C. felis* infection, by Chi-square test, Fisher's exact test and Z test. Statistical analysis was performed using SPSS (Version 16.0; SPSS Inc., Chicago, USA). P-value less than 0.05 was considered statistically significant.

RESULTS

In the present study, 152 cats were tested for the presence of *C. felis pmp*1 specific gene using the PCR technique. Out of 152 samples, 35 (23.03%) were positive (95% CI:16.30-29.70). The 96 cats (63.16%) were clinically healthy, while the remaining (n=56, 36.84%) had clinical signs of ocular or respiratory diseases. The obtained results showed a significant difference between cats that suffered from ocular or respiratory disorders (44.64%; 25 out of 56) and healthy cats (10.42%; 10 out of 96; P=0.01). Conjunctivitis and ocular discharge were the most common signs of *C. felis* infections (82.86%, 29 out of 35). There was also a significant association between conjunctivitis and PCR detection of *C. felis* (P=0.004). The *C. felis* was detected in only six cats with rhinitis and no symptoms of conjunctivitis. The prevalence was significantly higher in cats younger than one year (34.12%; 29 out of 85), compared to those older than one year (8.96%; 6 out of 67; P=0.02). The possibility of infection in cats younger than 1 year was detected 5.2 more than cats above 1 year (95% CI: 2.034-13.626). No significant difference was noted between the healthy and infected cats in terms of gender (25.45%; 14 out of 55 in males and 21.65%; 21 out of 97 in females), breed (23.81%; 30 out of 126 in DSH and 19.23%; 5 out of 26 in Persian), and lifestyle (22.22%; 28 out of 126 companion and 26.92%; 7 out of 26 feral; P>0.05). Results are summarized in tables 2 and 3. Some of the *C. felis* positive and negative samples are shown in Figure 1.
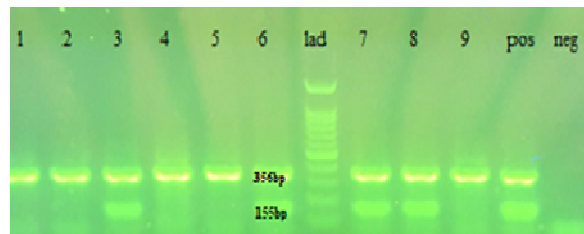

Figure 1. Agarose gel electrophoresis of the doubleplex PCR products for detection of *pmp*1 of *Chlamydophila felis* and *cytb* gene of *Felis catus* (Lanes 1 through 9, clinical samples; lane lad, 100bp ladder; lane positive and negative, positive and negative controls, respectively), Samples 3, 6, 7, and 8 included in *Cp. felis* positive samples (155bp band) while samples 1, 2, 4, 5, and 9 compromised negative for *Cp. felis* but positive for *cytb* gene (356bp band).

DISCUSSION

The present study revealed that 23.03% of companion and feral cats in Ahvaz were positive for *C. felis* using PCR technique. A significant association between PCR detection and conjunctivitis of *C. felis* was indicative of the fact that the presence of a pathogen is associated with the disease. The PCR is typically used to detect *C. felis* specific target genes in the conjunctiva. Consequently, the implementation of highly sensitive methods may provide an answer for the relatively high prevalence of *C. felis* infection. It is of utmost importance to detect *C. felis* infection since this pathogen is highly contagious and there are many feral cats in this area. The obtained results indicated that *C. felis* may be as a cause of ocular (conjunctivitis) or respiratory diseases in cats of the investigated region. These animals can be concerned as the agents of disease transmission, particularly to companion cats. It is essential to conduct studies on infectious and contagious diseases, such as Chlamydiosis since there is an increasing tendency among individuals to keep pets in their house. Moreover, some individuals bring feral cats into their homes, which is a matter of concern. Since the vaccination for *C. felis* is not often available in Iran, the principle of hygiene and avoidance of contact with cats, especially feral cats, are the only ways of disease prevention. Moreover, *C. felis* vaccines may lead to atypical reactions, such as fever, anorexia, lethargy, lameness (Sykes and Greene, 2012).

Numerous epidemiological surveys have reported the prevalence of *C. felis* in cats in different districts. For example, Millán and Rodríguez (2009) stated 27% seroprevalence in the wild cats of Europe. Halánová et al. (2011) reported a 45.16% prevalence of *C. felis* in cats in Slovak. The overall prevalence of *C. felis* exposure was estimated at 23.03% in cats of Ahvaz, which is lower than those rates mentioned in the above studies. On the other hand, the rates of infection were 11.5, 20, 17, and 5.9% in Australia (Sykes et al., 2001), northern Italy (Rampazzo et al., 2003), Canada (Sandmeyer et al., 2010), and in Lanzhou of China (Wu et al., 2013), which has a lower prevalence than the present study. The differences in the prevalence of *C. felis* exposure in cats can be associated with some factors, including geographical and ecological factors, presence of clinical illness, age of cats, and employed techniques of diagnosis. *Chlamydia* prevalence rates are usually higher in the summer months (Sykes and Greene, 2012; Borel et al., 2018). In a study conducted in Iran, Momtaz et al. (2014) reported that 40 cats (17.85%) out of 224 cases were infected with *C. felis* in PCR test. Maazi et al. (2016) showed that 20% of cats in Tehran were infected to *C. felis*. In the present study, the rate of infection was 23.03% representing a serious danger for the population of cats in Khuzestan Province. In this regard, it is essential to implement necessary testing techniques and therapeutic procedures of infected cats, and pay more attention to the preventive methods, such as isolation of patient cats and vaccination. It should be noted that the sensitivity and specificity of PCR are more than other available methods. The target genes in most studies are the outer membrane protein gene, 23S rRNA gene, and inclusion membrane protein A gene (Okuda et al., 2011). The complete genome sequence of *C. felis* contains an 1166-kb circular chromosome, encoding 1005 protein-coding genes, and a 7.5-kb circular plasmid (Sykes and Greene, 2012). Several risk factors affect the prevalence of *C. felis* infections, including age, breed,

Table 1. Properties of the employed primers (names, target gene, sequence, and references) in the PCR technique

Primer names	Target gene GenBank Accession No.	Sequences of Primers	Reference
*Kd1*F *Kd1*R	AB194813.1	5'-ATGAAACTTCGGCTCCCTTC-3' 5'-GGTTGGTGATTACGGTTGCT-3'	Canteki et al, 2014
*Cfpmp1*F *Cfpmp1*R	EF092092.1	5'-GGCGATCCCTATGTTGAGAA-3' 5'-CCACCGAAACACCCTGTAGT-3'	

Table 2. Prevalence of *C. felis* infection based on gender, breed, age, and lifestyle in cats of Ahvaz, Iran

Category	Groups	Prevalence	Odds Ratio	95% CI for OR	P-Value
Gender	Male	25.45% (14/55)	1.48	1.24- 2.68	0.5
	Female	21.65% (21/97)			
Breed	DSH	23.81% (30/126)	1.31	0.46-3.78	0. 6
	Persian	19.23% (5/26)			
Age	≤ 1 years	34.12% (29/85)	5.26	2.03-13.63	0.001
	> 1 years	8.96% (6/67)			
Life style	Feral	26.92% (7/26)	0.77	0.29-1.36	0.06
	Companion	22.22% (28/126)			

Table 3. Frequency distribution of *C. felis* based on clinical findings in cats in Ahvaz, Iran

PCR / Clinical findings	Positive	Negative
Positive	25 (44.64%)	31 (55.36%)
Negative	10 (10.42%)	86 (89.58%)
Total	35	117

gender, lifestyle, and health status (clinical signs particular conjunctivitis). According to the obtained results of the current study, it can be concluded that a significant percentage of cats infected with *C. felis* have clinical signs. Conjunctivitis and ocular secretion were the most common symptoms of *C. felis* infections (82.86%). Other symptoms include chronic conjunctivitis, chemosis, blepharospasm, and minor respiratory signs. The *C. felis* was detected in only six cats with rhinitis and no symptoms of conjunctivitis indicating that *C. felis* might cause URTD and not conjunctivitis. This finding is in line with previous studies conducted by Maazi et al. (2016) in which conjunctivitis was the most common clinical signs in the cats infected with *C. felis* (37.1%). Concurrent feline immunodeficiency virus infection in cats prolonged the duration of conjunctivitis and clinical signs noted after the ocular inoculation of *C. felis* (Sykes and Greene, 2012). Therefore, it is essential to use the samples of nasal and pharyngeal to increase the isolation rates of this pathogen. The performance of sampling techniques at different time points can cause distinct *C. felis* isolation rates. Vigorous swabbing of mucosal surfaces is essential to obtain enough epithelial cells containing the organism. In an experimental study on 26 *C. felis*-infected cats, the pathogen was detected in the conjunctiva of all infected cats 3 days after inoculation (Masubuchi et al., 2002). Conjunctivitis is one of the common reasons for hospital referrals accounting for up to 3% of cats in the UK and USA (Sykes and Greene, 2012). For the first time, Sibitz et al., 2011 isolated the known human microorganism *C. pneumoniae* in feline conjunctivitis cases. In the present survey, the systemic signs of fever, lethargy, lameness, and weight loss were not observed in the investigated cats. Moreover, there was a significant difference between the clinical findings and PCR results (44.64% of cats with symptoms were positive, while the infection was identified only in 10.42% of healthy cats). In the present study, a higher prevalence of *C. felis* infection was detected in cats younger than 1 year (34.12%), compared to cats older than 1 year

(8.96%). Therefore, there is an age-related propensity in the infected cats, meaning the cats younger than 1-year-old are more susceptible to persistent or recurrent infection. The prevalence of *C. felis* infection is low in kittens younger than 2 months of age, probably due to their passive immunity. Serologic surveys in cats coupled with organism culture or genetic detection have indicated that the older the cat, the more increased the immunity (Sykes and Greene, 2012). Furthermore, surveys using both PCR and culture emphasized that cats younger than 1 year of age were the most likely to be infected with Chlamydia (Sykes, 2005). In the present study, the prevalence of *C. felis* was higher in male cats (25.45%); however, statistical analysis did not show any significant difference. This means that *C. felis* exposure has no gender predilection in the cats, which was consistent with previous studies (Sykes and Greene, 2012). Wu et al. (2013) indicated that the prevalence of *Cp. felis* infection in feral cats (14.3%) was higher than household cats (3.9%). In the current study, the prevalence was higher in feral cats (26.92%) than companion ones (22.22%); however, the difference was not significant statistically. These results may refer to fewer cases of infected cats in the feral group compared with companion cats. In our study, the samples were collected from December 2016 to October 2017. The obtained results provide useful information for future studies. In conclusion, it can be stated that the PCR technique is a sensitive and specific method for the detection of *C. felis*. This study highlights the necessity of using rapid and effective diagnostic techniques for screening healthy and ill cats. The findings of the current study showed that *C. felis* is a specific infection and appears to be endemic in cats of Ahvaz. The *C. felis* is a zoonotic microorganism, which highlights the importance of the quick treatment of the infected cats. In this regard, testing programs for the diagnosis and prevention of contact are the most effective preventative ways for sick and healthy animals. Vaccination has proven to be an effective strategy to reduce *C. felis* infection and may potentially eliminate it. As a result, a prolonged course of

tetracycline or doxycycline is necessary to eradicate the infection. To evaluate the response to the treatment, the concurrent presence of viral causes of feline respiratory disease should be considered. Further studies are necessary for the comparison of PCR and culture results in naturally infected cats.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article. All procedures which might be associated with discomfort, including conjunctival swabs, were performed by an experienced veterinarian.

Conflict of Interest

The authors declare that they have no conflict of interest.

Grant Support

References

Bannasch, M.J., Foley, J.E., 2005. Epidemiologic evaluation of multiple respiratory pathogens in cats in animal shelters. J Feline Med Surg 7, 109-119.

Bommana, S., Pokinghorne, A., 2019. Mini Review: Antimicrobial control of chlamydial infections in Animals: Current practices and issues. Front Microbiol 10, 1-9.

Borel, N., Polkinghorne, A., Pospischil, A., 2018. A review on chlamydial diseases in animals: still a challenge for pathologists? Vet pathol 55, 374-390.

Cantekin, Z., Solmaz, H., Altug, N., Ozmen, G.O., 2014. Development of pmp Gene-Specific PCR Assay with A Host Specific Internal Control for *Chlamydophila felis*. Thai J Vet Med 44, 469.

Halánová, M., Sulinova, Z., Čisláková, L., Trbolova, A., Páleník, Ľ., Weissova, T., *et al.*, 2011. *Chlamydophila felis* in Cats–Are the Stray Cats Dangerous Source of Infection? Zoonoses Public Hlth 58, 519-522.

Hartmann, A.D., Hawley, J., Werckenthin, C., Lappin, M.R., Hartmann, K., 2010. Detection of bacterial and viral organisms from the conjunctiva of cats with conjunctivitis and upper respiratory tract disease. J Feline Med Surg 12, 775-782.

Helps, C., Reeves, N., Egan, K., Howard, P., Harbour, D., 2003. Detection of *Chlamydophila felis* and feline herpesvirus by multiplex real-time PCR analysis. J Clin Microbiol 41, 2734-2736.

Johnson, F., 1984. Isolation of *Chlamydia psittaci* from nasal and conjunctival exudate of a domestic cat. Vet Rec 114, 342-344.

Kang, B.-T., Park, H.-M., 2008. Prevalence of feline herpesvirus 1, feline calicivirus and *Chlamydophila felis* in clinically normal cats at a Korean animal shelter. J Vet Sci 9, 207-209.

Maazi, N., Jamshidi, S., Kayhani, P., Momtaz, H., 2016. Occurrence of *Chlamydophila felis*, feline herpesvirus 1 and calcivirus in domestic cats of Iran. Iran J Microbiol 8, 312.

Masubuchi, K., Nosaka, H., Iwamoto, K., Kokubu, T., Yamanaka, M., Shimizu, Y., 2002. Experimental infection of cats with *Chlamydophila felis*. J Vet Med Sci 64, 1165-1168.

Millán, J., Rodríguez, A., 2009. A serological survey of common feline pathogens in free-living European wildcats (Felis silvestris) in central Spain. Eur J Wildlife Res 55, 285-291.

Momtaz, H., Alirezaie, M., Keyhani, P., 2014. Molecular detection of *Chlamydophila felis* in ocular discharge of domestic cats in Tehran & Isfahan. Biological Journal of Microorganism 3, 49-57.

Okuda, H., Ohya, K., Shiota, Y., Kato, H., Fukushi, H., 2011. Detection of *Chlamydophila psittaci* by using SYBR green real-time PCR. J Vet Med Sci 73, 249-254.

Rampazzo, A., Appino, S., Pregel, P., Tarducci, A., Zini, E., Biolatti, B., 2003. Prevalence of *Chlamydophila felis* and feline herpesvirus 1 in cats with conjunctivitis in northern Italy. J Vet Intern Med 17, 799-807.

Sandmeyer, L.S., Waldner, C.L., Bauer, B.S., Wen, X., Bienzle, D., 2010. Comparison of polymerase chain reaction tests for diagnosis of feline herpesvirus, *Chlamydophila felis*, and Mycoplasma spp. infection in cats with ocular disease in Canada. Canadian Vet J 51, 629-633.

Sibitz, C., Rudnay, E.C., Wabnegger, L., Spergser, J., Apfalter, P., Nell, B., 2011. Detection of Chlamydophila pneumoniae in cats with conjunctivitis. Vet Ophthalmol 14, 67-74.

Sykes, J.E., 2005. Feline chlamydiosis. Clin Tech Small An P 20, 129-134.

Sykes, J.E., Allen, J.L., Studdert, V.P., Browning, G.F., 2001. Detection of feline calicivirus, feline herpesvirus 1

and Chlamydia psittaci mucosal swabs by multiplex RT-PCR/PCR. Vet Microbiol 81, 95-108.

Sykes, J.E., Greene, C.E., 2012. Chlamydial infections. In: Greene, C.E. (Ed.), Infectious Diseases of the Dog and Cat, Missouri, St. Louis, pp. 270-276.

Wu, S.-M., Huang, S.-Y., Xu, M.-J., Zhou, D.-H., Song, H.-Q., Zhu, X.-Q., 2013. *Chlamydia felis* exposure in companion dogs and cats in Lanzhou, China: a public health concern. BMC Vet Res 9, 104.