# Occurrence of *Chlamydophila felis* in a cattery in Osasco city, São Paulo state

## *Ocorrência de Chlamydophila felis em um gatil na região de Osasco, São Paulo*

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**Abstract**

*Chlamydophila felis* is associated with upper respiratory tract infections. In the present study, 31 cats from a noncommercial shelter located in Osasco, SP, Brazil, were examined. The cats presented with clinical manifestations, which were classified from grade 1 to 4, with 4 indicating severe manifestations. In total, 16.13% of the cats presented with grade 1 severity of clinical manifestations, 25.81% presented with grade 2, 38.71% presented with grade 3, and 19.35% presented with grade 4. PCR was used to detect *C. felis* in samples taken from the oral mucosa and ocular conjunctiva of both eyes using sterile, dry cotton swabs. Overall, 58% of the samples were positive for *C. felis.* Of these animals, none showed clinical manifestations that were classified as grade 1, whereas 5.56% of cats were classified as grade 2, 61.11% were classified as grade 3, and 33.33% were classified as grade 4. The median clinical manifestation intensity score for the first group was 3 and ranged from 2 to 4. In the second group not positive for *C. felis*, 38.45% of the animals (5/13) presented with manifestations classified as grade 1, 53.85% (7/13) were classified as grade 2, 7.69% (1/13) were classified as grade 3, and no animals were classified as grade 4. The median clinical manifestation intensity score for the second group was 2 and ranged from 1 to 3. In this study, there was a high occurrence of *C. felis* in animals with clinical manifestations.

**Keywords:** *Chlamydophila felis.* Shelter. Upper Respiratory Disease (URD).

**Resumo**

A *Chlamydophila felis* está associada à infecção de trato respiratório superior. No presente estudo, foram utilizados 31 felinos de um gatil não comercial, localizado em Osasco/SP. Os gatos apresentavam manifestações clínicas, classificadas de 1 a 4, sendo 4 atribuído àqueles que apresentavam pior manifestação clínica. Foi observada a intensidade de manifestação clínica grau 1 em 16,13% dos gatos, a 2 em 25,81%, a 3 em 38,71% e a 4 em 19,35%. A detecção de *C. felis* foi realizada por técnica de PCR em amostras obtidas com suabes de algodão, seco e estéril, de mucosa oral e de conjuntiva ocular de ambos os olhos. Verificou-se que 58% das amostras para *C. felis* foram positivas, entre esses animais, nenhum apresentou manifestação clínica classificada como grau 1, o grau 2 foi observado em 5,56% dos gatos, 61,11% para o 3 e 33,33% dos animais apresentava a intensidade 4. Verificou-se que para o primeiro grupo a mediana dos escores de intensidade das manifestações clínicas observadas foi de 3, variando de 2 a 4. No segundo grupo, foi observado 38,45% (5/13) dos animais para a intensidade 1, 53,85% (7/13) para a 2 e 7,69% (1/13) para a 3, nenhum animal deste grupo apresentou o grau 4. Verificou-se para o segundo grupo, a mediana dos escores de intensidade das manifestações clínicas observadas foi de 2, variando de 1 a 3. Neste trabalho foi observada uma elevada ocorrência de *C. felis* em animais com manifestação clínica.

**Palavras-chave:** *Chlamydophila felis.* Gatil. Infecção de Trato Respiratório Superior (ITRS).

## Introduction

Feline chlamydiosis is a contagious infectious disease that is responsible for causing acute and chronic conjunctivitis in cats suffering from an upper respiratory disease (URD). URD in cats is very common in animals living in shelters, has a high

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morbidity rate and can, in some cases, be fatal. The high predisposition to URD in shelters is due to the high turnover of the population, high density, stress, prior lack of veterinary care, inadequate nutrition and recurring morbidity (BANNASCH; FOLEY, 2005).

Baker (1942) described the first case of *Chlamydophila felis,* formerly called *Chlamydia psittaci* variant *felis*; the taxonomic revision was undertaken in 1999 (EVERETT; BUSH; ANDERSEN, 1999). In the 1950s, feline calicivirus and feline herpes virus type 1 were discovered and linked to URD. Thus, chlamydiosis was no longer the only causative agent of URD, but it has a specific role as the causative agent of acute and chronic conjunctivitis in cats (HARBOUR; HOWARD; GASKELL, 1991).

*Chlamydophila felis* is an obligate intracellular bacterium that seems to have a predilection for conjunctival epithelial cells. Transmission likely occurs through close contact with infected cats, either through aerosols or fomites (HALÁNOVÁ et al., 2011). Intense contact with an infected animal is required for transmission between cats, particularly with ocular secretions, which are likely the most relevant secretions in the process. The highest occurrence has been shown to occur in cats less than one year old. The incubation period for *C. felis* is between two and five days (GRUFFYDD-JONES et al., 2009).

In general, chlamydial infections tend to follow a chronic and insidious disease course, progressing to stages without clinical manifestation. It is unclear whether the chronic disease results from repeated reinfection or a single persistent chlamydia infection (GRUFFYDD-JONES et al., 2009).

The pathogenesis of *C. felis* is not yet fully understood, and there are very few studies that have examined feline chlamydiosis. The occurrence of this disease has been reported in some epidemiological studies, such as Holst et al. (2006), who demonstrated that the prevalence of *C. felis* in cats in Sweden was 15%, whereas Sykes et al. (1999) reported a prevalence of 14.3% for *C. felis* in Australia.

In Brazil, the first study on feline chlamydiosis was conducted in 2008 on domestic cats from five municipalities in the northeastern region of the state of São Paulo. In that study, *C. felis* was detected in 6% of the samples analyzed (SEKI, 2008). Previous studies have examined cats from veterinary hospitals and private clinics.

Taking into consideration the growing number of domestic cats confined to urban areas, studies on the prevalence of this agent in the cat population can help to define the importance of *C. felis* as a causative agent of disease.

This study aimed to determine by PCR the occurrence of *Chlamydophila felis* in eye conjunctiva and oral swabs of cats from a cattery of Osasco city, São Paulo State.

## Materials and Methods

For this study, sterile and dry swabs were used to sample the ocular conjunctiva and mouths of 31 cats housed at a noncommercial cat shelter located in the city of Osasco, SP, Brazil. This population consisted of cats of unknown origin that were collected from public roads. The animals did not have a defined breed and were young, between two months and five years of age. An estimated 16% of the animals were younger than one year of age. In the shelter, 52% of the cats (16/31) were male, and 48% (15/31) were female.

The animals were classified based on the intensity of their clinical manifestations, which ranged from 1 to 4 (from least severe to most severe). Thus, the animals classified as a 1 presented with the mildest manifestations, including mild ocular and/or nasal serous secretions. Animals classified as a 2 presented with moderate mucopurulent ocular and/or nasal secretions. Animals classified as a 3 presented with severe mucopurulent ocular and/or nasal secretions, and the animals classified as a 4 presented with more severe presentation, including severe mucopurulent ocular and/or nasal secretions, erythema, edema and lesions (uveitis, hyphema and/or ulcer).

## Detection of *Chlamydophila felis* by PCR

Total DNA was extracted from the swabs using the commercial kit DNeasy Tissue (Qiagen, Crawley, UK) according to the manufacturer's instructions. The PCR reactions contained 0.2 µM of each primer (Table 1), 1X buffer, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, 0.625 U Platinum® TaqDNA Polymerase (Life Technologies, Brazil), 5 µl of the sample DNA and ultrapure water to a final volume of 25 µl. The reaction conditions were as follows: 1 cycle at 94ºC for 3 min, 35 cycles of 94°C for 45 s, 55ºC for 30 s and 72ºC for 45 s and 1 cycle at 72°C for 7 min (HELPS et al., 2001). The vaccine Felocell® CVR-C (Pfizer) was used as a positive control. Autoclaved ultrapure water was used as a negative control for the reactions.

The amplification product and a molecular weight marker were analyzed via gel electrophoresis on a 1.5% agarose gel containing 0.1 µg ethidium bromide per ml and visualized on an ultraviolet light image capturing system. Samples with bands corresponding to 129 bp were considered positive.

Statistical analyses were performed, including Spearman's correlation and the chi-squared test using the program GraphPad INSTAT 1992-98.

## Results

Clinical manifestations were observed in all of the animals housed in the shelter. In total, 16.13% of the cats (5/31) presented with an intensity of manifestations classified as grade 1, 25.81% (8/31) presented as grade 2, 38.71% (12/31) presented as grade 3, and 19.35% (6/31) presented as grade 4.

Among the 31 animals surveyed in the non-commercial shelter, *C. felis* was detected in 58.06% of the animals (18/31).

The animals were divided into two groups: the first group consisted of 18 animals in which *C. felis* was detected, and the second group consisted of 13 animals in which the pathogen was not detected. In the first group, none of the animals presented with an intensity of manifestations classified as grade 1, 5.56% of the animals (1/18) were classified as grade 2, 61.11% (11/18) were classified as grade 3, and 33.33% (6/18) were classified as grade 4 (Table 2). In the first group, the median score for the intensity of the clinical manifestations was 3 and ranged from 2 to 4. In the second group, a grade 1 intensity was observed in 38.45% of the animals (5/13), 53.85% (7/13) presented with grade 2, and 7.69% (1/13) presented with

Table 1 – PCR primers used to detect *C. felis**

| Primers | Orientation | Sequence (5'-3')• | Region of the gene MOMP |
|---|---|---|---|
| Chl rev | Anti-sense | TCCTAAAAGAGTTGGGTTCCAGG | 965-986 |
| Chl for | Sense | ATGCTTGTTCCATACATTGGGG | 1071-1093 |

• GenBank sequence accession X61096, MOMP=major outer membrane protein.

* Helps et al. (2001).

Table 2 – Intensity of clinical manifestations and the presence or absence of *C. felis* - Osasco, SP, Brazil - 2012

| Intensity of manifestations | *C. felis* present | | *C. felis* absent | |
|---|---|---|---|---|
| 1 | 0 | 0% | 5 | 38.46% |
| 2 | 1 | 5.56% | 7 | 53.85% |
| 3 | 11 | 61.11% | 1 | 7.69% |
| 4 | 6 | 33.33% | 0 | 0% |
| Total | 18 | | 13 | |

The median score for group 1 was statistically higher than the median score for group 2 ($p < 0.0001$).

grade 3. None of the animals in this group presented with grade 4 (Table 2). For the second group, the median score for the intensity of the clinical manifestations was 2 and ranged from 1 to 3.

## Discussion

Some epidemiological studies have reported the occurrence of *C. felis* in veterinary hospitals and clinics. In Sweden, 15% of the animals studied were infected (HOLST et al., 2006), whereas in Australia, the prevalence was 14.3% (SYKES et al., 1999). In Brazil, Seki (2008) found a prevalence of 6% in cats.

This study aimed to determine the prevalence of *C. felis* in an animal shelter using PCR. The shelter analyzed had a high density of cats of unknown origin and also had a history of recurrent upper respiratory disease and acute and chronic conjunctivitis in the cats. Upon collection, all of the cats presented with clinical manifestations. The ocular conjunctiva and oral mucosa swabs were obtained during June and July 2012, a period in which there is a higher frequency and evidence of clinical manifestations of URD in the southern hemisphere.

Thus, the present study found a high occurrence (58.06%) of *C. felis* in the shelter compared with previous studies. These data suggested that a high density of animals could be associated with the spread of the agent in the clinical evolution of the disease.

All of the cats in the study had clinical manifestations of URD, and in the animals that tested positive for *C. felis,* the median disease score was 3 and ranged from 2 to 4, indicating that most of the cats presented with severe mucopurulent ocular and/or nasal secretions. For animals in which *C. felis* was not detected, the median score was 2 and ranged from 1 to 3, indicating that most of the cats presented with moderate mucopurulent ocular and/or nasal secretions. Given that there was a statistically significant difference between the two groups ($p < 0.0001$), the presence of *C. felis* was associated with a level of more intense clinical manifestations.

Six months after the date of collection, 19.35% of the animals (5/31) in the first group had clinical manifestations that worsened, resulting in the animals' death; all 5 of these cats had grade 3 or 4 manifestations. In contrast, none of the cats in the second group died, confirming that the disease severity was worse for the cats in which *C. felis* was detected.

This study demonstrated the importance of *C. felis* in catteries of high population density, although there is no scientific evidence of the zoonotic potential of this pathogen. Due to the interspecies transmission inter of *C. psittaci* from birds to humans that occurs (EIDSON, 2002), catteries should take sanitary control of *C. felis* and use precautions in managing of cats with conjunctivitis.

## Conclusion

In the present study, there was a high frequency of *C. felis* detected in cats with clinical manifestation, especially in animals in the shelter that had the most severe clinical presentations. Thus, further studies of cat shelters are needed to determine the real significance of *C. felis* in causing disease in cats that live in conditions with a high population density.

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