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Chlamydophila felis ANTIBODIES IN STRAY DOMESTIC SHORT-HAIRED CATS (*Felis catus*) FROM A TERTIARY PUBLIC HOSPITAL

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ABSTRACT

Forty-two stray domestic short haired cats (*Felis catus*), of both sexes and various ages (four to 48 months), were captured within a tertiary public hospital in Metro Manila and used in the study. These animals had no deworming and vaccination records. Convenience sampling was done by working in a specified time frame set prior to the study. Routine physical examination was done and blood samples were collected from the animals and tested against *Chlamydophila felis* antibodies using a commercially available ELISA antibody test kit. The qualitative and quantitative classifications of the results were acquired through digital image scanner software that uses a TWAIN compliant scanner. Of the samples tested, one (2%) female adult cat had a titer of >1:32 and was interpreted as positive. Two (5%) were suspicious with a titer of ≤1:16 and 39/42 (93%) had no serologic evidence of exposure to *Cp. felis*. Spearman's Test of Independence showed no significant correlation between the animal's sex and age to the presence of antibodies against *Cp. felis.* This study shows that stray domestic short haired cats within the vicinity of a certain tertiary public hospital are exposed to *Chlamydophila felis*.

Key words: cat, *Chlamydophila felis*, domestic, hospital, public

INTRODUCTION

A tertiary public hospital is a facility which provides a tertiary hospital services, with teaching, training and research functions (Villar, 2010). The location where the stray cats were obtained from the study serves as a national tertiary referral center and teaching hospital. The presence of relatively dense number of stray domestic short-haired cats moving freely in and out of this hospital, where people are mostly immunocompromised, favors the spread of zoonotic diseases including *Chlamydophila felis* (*Cp. felis*) infection.

Cp. felis was formerly classified as *Chlamydia psittaci* and *Chlamydia psittaci var felis* (Wills, 1986; Gruffydd-Jones *et al*., 1995; McDonald *et al*., 1998; TerWee *et al*., 1998; Sparkes *et al*., 1999) until Everett (2000) reclassified the taxonomy of family Chlamydiaceae and is now named as such. *Cp. felis* is an obligate, highly specialized intracellular, Gram negative, rod-shaped coccoid bacterium that lacks peptidoglycan in the cell wall (Vanrompay *et al*., 1995; Holst, 2005; Gruffydd-Jones *et al.,* 2009). It has been recognized as an important infectious agent in cats which serve as the definitive host (Gunn-Moore *et al.,* 1995).

Infected cats exhibit signs of upper or lower respiratory tract disease, a condition known as feline pneumonitis (Hamre and Rake, 1944), and is related to possible atypical pneumonia in humans (Baker, 1942; Trávnicek *et al.,* 2002). It was later identified also as an ocular pathogen, which results in conjunctivitis and ocular discharges in cats and has been considered as the primary and most important etiologic agent of feline upper

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respiratory tract infections (Trávnicek *et al.,* 2002). *Cp. felis* requires close contact between an uninfected cat and a cat with ocular discharge. The ocular discharge is probably the most probable important body fluid for infection (Gruffydd-Jones *et al.,* 2009).

Search for similar studies revealed that although several local prevalence studies had already been conducted, none has been published. Furthermore, this is the first study conducted to determine the presence of *Cp. felis* antibodies in stray domestic short haired cats in a tertiary public hospital. The results of the study will provide the initial report of the presence of *Cp. felis* in a tertiary public hospital. It is the hope of the authors that preventive and control measures will be made in order to minimize the possibility of infection and disease outbreak. Lastly, the study hopes to pave the way for better hygiene and management practices that will prevent the spread of the disease from animals to humans.

MATERIALS AND METHODS

Approximately sixty (60) stray domestic short haired cats were seen during an initial ocular inspection conducted in different areas within a tertiary public hospital in Metro Manila. Inquiries from security personnel and hospital staff confirmed that the diet of these animals includes, but not limited to, table scraps from the hospital kitchen, rodents and small birds. Moreover, these cats had no history of deworming and vaccination. Convenience sampling was done on specific pre-selected dates within an operating time-frame (six o'clock in the afternoon to 12 midnight).

Forty-two (42) stray domestic short haired cats were caught using a drop trap as described by Reyes *et al*. (2013). The captured stray cats were transported and temporarily housed individually at the Department of Veterinary Clinical Sciences, University of the Philippines Los Baños, Laguna. Commercial dry cat food and water were provided throughout the duration of the confinement. The sex (14 males and 28 females) and age (14 juveniles and 28 adults) of the cats were noted. The latter was determined by examination of the dental arcades. Cats whose molars have not yet erupted were considered less than six months of age (juvenile) while those with erupted molars were considered adults with at least 6 months of age (Crossley *et al*., 1995).

The procedures described in this study have been approved by the Institutional Animal Care and Use Committee (IACUC) of the University of the Philippines Los Baños. The stray cats were humanely destroyed after the study.

The cats were chemically restrained using tiletamine hypochloride and zolazepam hypochloride (Zoletil® 50mg/ml, Virbac Laboratories, Carros, France) at a dose of 5mg/kg given intramuscularly using a 3cc sterile disposable syringe with a 23G hypodermic needle. Blood samples were collected via jugular venipuncture using a 23G hypodermic needle attached to a 3cc sterile disposable syringe. Blood samples were immediately transferred to clean, properly-labelled 4ml vacuum tubes without anticoagulant (red-capped) and allowed to stand at room temperature for at least an hour. The test tube was "rimmed" to free the clots and the samples were centrifuged at the recommended rate of 3,000 revolutions per minute for 5 minutes to allow maximum serum extraction. The serum was transferred in several aliquots into clean, properly labelled Eppendorf tests using a sterile disposable tuberculin syringe per sample.

The sera were tested for *Cp. felis* using a commercially available ELISA antibody detection kit (Feline *Toxoplasma gondii* and *Chlamydophila felis* Antibody Test Kit, ImmunoComb®, Biogal Laboratories, Galed, Israel) following the manufacturer's instructions. The resulting antibodies are displayed as a change in color within a "comb's tooth". The results were interpreted using digital scanning software (CombScan®2000; Biogal Laboratories, Galed, Israel) with the results expressed as titers. This software

provides an objective quantitative (titre levels) & qualitative (net absorbance) results of the ELISA test kit by employing a TWAIN (Technology Without An Interesting Name) compliant scanner to scan the developed comb. The software is an image analyzer that detects the faintest spots on the comb that are barely visible to the naked eyes, thereby, providing a more accurate interpretation of the test results. Once the spot is detected, it is quantified and assigned a mean net grey level on a scale of 0 and 255 (the net absorbance). Subtracting the background color from the color level of the spot derives a quantitative value and a qualitative classification.

The results acquired from the software are interpreted as: *Negative* result (no net absorbance) which has an antibody titer of 0, meaning that there is undetectable antibody levels to *Cp. felis*; *Suspicious* result is when the animal has an antibody titer of ≤1:16, meaning there is no significant serologic evidence of *Cp.felis* infection; *Positive* result is with those antibody titers ranging from 1:32 to 1:64 and are interpreted as animals with serologic confirmation of *Cp. felis* infection and; *High positive* results are those with antibody titers of ≥1:128 and are interpreted as animals with serologic confirmation of *Cp. felis* infection.

The correlation of sex and age to the antibody titer of *Cp. felis* were analyzed using Spearman's Test of Independence.

RESULTS AND DISCUSSION

Forty-two stray domestic short haired cats found in the vicinity of a tertiary public hospital were tested for the presence of *Cp. felis* antibodies. Most of these stray animals have been observed behind the buildings where hospital wastes, including kitchen left overs, are temporarily placed and inside the hospital buildings where close contact with patients have been observed.

In this study, the developed comb was scanned using a digital image scanner software that employs a TWAIN compliant scanner. Based from the results of the digital image scanner software, only one (2.38%) of these cats had a titer of >1:32 thus tested positive while, two (4.46%) have a titer of ≤1:16, or suspicious for antibodies against *Cp. felis* (Table). Furthermore, the cat that tested positive (1/42) and suspicious (2/42) for *Cp. felis* antibodies were all adult female cats.

The female cat that was exposed to *Cp. felis* exhibited signs of conjunctivitis, a characteristic sign of *Cp. felis* infection (Figure 1). On the other hand, bilateral ocular mucous discharge with conjunctivitis was observed in cats that tested suspicious (Figure 2). Conjunctivitis is the inflammation of the conjunctival tissues (Davidson, 2008). The

Table. Frequency distribution based on age and sex of stray domestic short haired cats (n=42) found within the vicinity of a tertiary public hospital, tested for the presence of *Chlamydophila felis* antibodies using an ELISA antibody kit.

Qualitative classification	Quantitative IgG titer	Frequency				Total (%)
		Male		Female		
		Juvenile	Adult	Juvenile	Adult	
Negative	0	7	6	7	19	39 (93%)
Suspicious	≤ 1:16	0	0	0	2	2 (5%)
Positive	1:32 – 1:64	0	0	0	1	1 (2%)
Total		7	6	7	22	42 (100%)

Figure 1. Representative picture of an adult female cat that was qualitatively classified as positive. Note the conjunctivitis on the left eye.

Figure 2. Representative picture of an adult female cat that was qualitatively classified as suspicious. Bilateral ocular mucous discharge and conjunctivitis on its right eye were observed.

conjunctiva is the mucous membrane lining the posterior aspect of the upper and lower eyelids (palpebral conjunctiva), both surfaces of the third eyelid, and the anterior sclera (bulbar conjunctiva) (Aroch *et al*., 2008).

Nevertheless, not all affected cats would show conjunctivitis and thus absence of clinical sign does not prove the absence of infection. Sparkes *et al.* (1999) stated that clinical signs may resolve within three months in untreated cats but continue to excrete the *Cp. felis* infection for at least eight months.

The much lower positive result (2%) of this study may be due to the actual low occurrence or due to the variability in the number of samples tested. Previous unpublished studies conducted used a smaller sample size from a smaller study area. In this study, a larger sample size from a tertiary public hospital where these cats were acquired is larger by almost 50%. Transmission of *Cp. felis* is through direct contact. A larger area provides wider space for the cats to roam and mark their territories thus render lesser chances to acquire the infection from affected cats.

In contrast, higher occurrence rate have been recorded in studies abroad compared locally. In a study in United Kingdom, 30% of the cats with conjunctivitis showed positive result for *Cp. felis* (Sparkes, 1999). Moreover several studies on cats in different countries as cited by Sykes (2005) show different rates of positive evidence of *Cp. felis* antibodies: 14.3% (66/ 462) in Australia, 17.7% (20/ 113) in Great Britain, 20% (14/ 70) in Haiti, and 11.5% (26/ 266) in Switzerland.

On the other hand, 5% of the cats under study tested suspicious. According to Cohn (2006), the predominant antibodies in the early stages of infection are IgM-mediated which later switches to IgG and/ or IgA. Those that were presented as suspicious may be exhibiting the infection sub-clinically and thus cannot be detected by the commercially available ELISA test kit used in the study as it only detects IgG antibodies.

It is also possible that differences in the qualitative classification among the cats may be attributed to repeated exposure to *Cp. felis*. In this study, the cat that tested positive possibly had increased contact to cats shedding the agent as compared to cats that tested suspicious and negative.

Behavior of cats also plays a major role in the transmission of the disease. Adults

have higher tendency to roam in search for food as compared to juveniles; consequently, the former have higher risk of exposure from affected cats. Similarly, females are less aggressive and more sociable compared to males (Bendinelli *et al*., 1995). The former can coexist with other cats and therefore increasing the probability of disease transmission (Aiello, 2012). Furthermore, females undergo estrus, which may result in immunosuppression that favors disease to be acquired. Similarly, Gunn–Moore *et al*. (1995) stated that adult cats were significantly more likely to be seropositive than those that are less than 1 year of age.

In contrast, Sparkes *et al*. (1999) observed that *Cp. felis* more frequently infects kitten and young adults. This may be due to the decline in maternal antibodies during the 5th to 12th weeks of age. This was also confirmed in a study described by Sykes (2005) wherein cats less than 1 year old were most likely to be infected with *Cp. felis* while those that are 5 years and older were least likely to be infected.

Statistical analysis using Spearman's Test of Independence showed no significant correlation between the animal's sex and age to the presence of antibodies against *Cp. felis*. This may be due to only one cat that tested positive thus no generalization can be made.

The zoonotic potential of *Cp. felis* has been presented in the study of Sparkes *et al.* (1999) where two out of four cases provided strong evidence of transmission from cats to humans. Moreover, in a study by Miyashita *et al*. (2005), one patient had an antibody titer suggestive of *Cp. felis* infection. It is, therefore, recommended for the hospital personnel to undergo testing against *Cp. felis* infection.

It is also highly recommended that the domestic short haired cats that continuously inhabit the vicinity of the hospital be controlled and eliminated. Susceptibility to contract the agent was thought to be increased in areas where cats are living in cluster population (Trávnicek *et al.,* 2002) just like the significant number of domestic short-haired cats in this particular tertiary hospital. It has also been observed that these cats can roam throughout the hospital buildings where patients and hospital personnel reside. This provides the chance to contract the disease as it is transmitted through close contact with other infected animals, through aerosols and via fomites (Sykes, 2005).

One way to eliminate these domestic short haired cats from proliferating is through spay and neuter campaign within the vicinity of the hospital and its neighboring areas. Proper garbage disposal with more frequent collection schedule is also suggested to keep the cats from scavenging for food at the dump site.

Public notice and warnings should be enforced with information, education and communication campaign to the hospital personnel and general public. This is to increase awareness of the potential zoonotic risk of *Cp. felis* infection. Further, the public will be unhesitant to follow hospital's rules in not feeding cats if the public are well informed regarding the risk of the disease and how it can be contracted and prevented.

Nonetheless, proper hygiene through washing of hands with soap and water and sanitation by always keeping the surroundings clean should be exercised. If the public neglects these practices, it may possibly cause contamination from the body fluids of the affected cats (Trávnicek, 2002). The results of this study may not be able to generalize the seroprevalence of *Cp. felis* on a national level, however, these results may be used by the hospital administration to monitor the presence of the infection in the facility. It is also recommended that further epidemiological studies should be done in domestic short-haired cats found in other hospitals to assess the hazard to human health (Coulter and Longbottom, 2003).

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