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Molecular Detection of *Leptospira* spp. in Domestic Cats from Small Animal Hospital, Chiang Mai, Thailand

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Abstract: Domestic cats can transmit *Leptospira* spp. and may play an important role in human infection. The aim of this study was to detect Leptospiral infection in mature cats presented to Small Animal Veterinary, Chiang Mai University, by using immunofluorescence assay (IFA) and real-time PCR (RT-PCR) techniques. Eighty mature cat serum and urine were collected. The results showed that all of IFA's serological results were negative. Otherwise, we found 2.5 percent of outdoor cats (2/80) positive with RT-PCR, with one cat positive in urine and another positive in urine and serum from the Muang and Doi Tao areas, respectively. This study discovered the presence of *Leptospira* spp. in mature cats brought to Small Animal Veterinary, Chiang Mai University, implying that outdoor cats could be an important reservoir for the transmission of Leptospira bacteria. Furthermore, more research is needed to investigate the role of transmission in the cat and to examine the disease prevention rule not only for the owner but also for staffs in animal hospitals.

Keywords: Leptospirosis, Cats, Immunofluorescence assay, Real time-PCR, Chiang Mai

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Introduction

Leptospirosis is a serious zoonotic illness caused by the spirochaete bacterium Leptospira spp. in many countries, and it is considered an emerging or re-emerging disease. Pathogenic leptospirosis is classified as a single species, *Leptospira interrogan*, with over 200 serovars of pathogenic bacteria reported (Costa et al., 2015; Haake and Levett, 2015). Leptospirosis is most widespread in tropical and subtropical areas, such as Thailand, where the disease is a serious public health concern, with an average of 6.6 per 100,000 individuals afflicted each year and up to 25 per 100,000 during outbreaks (Hinjoy, 2016). This pathogen is spread through the urine of infected animals that has been contaminated by water, moist soil, or vegetables. Following wounds and scratches, the infection can enter the human body through the skin (Haake and Levett, 2015).

Many mammal species have been infected with leptospirosis and serve as primary reservoirs. Domestic animals, such as domestic cats and dogs, will be contaminated by rodents or environmental contamination. The symptoms of infected animals can be subclinical or mild, whereas the symptoms of an incidental host infected with the disease are clearly dependent on the type of animal and serovars of pathogens (Ellis, 2015). In animal, antibody prevalence of 24.8 percent in buffalos, 28.1% in cattle, and 11.3 percent in pigs have recently been recorded in Thai livestock (Chadsuthi et al., 2017). In addition, the prevalence of antibodies in dogs in Chiang Mai ranged from 11% to 19%. (Meeyam et al., 2006).

In the case of humans, an incidental host is discovered to be the source of infection from various animals. In Thailand, a large number of cats have outside access and are allowed to hunt prey. Cats can get *Leptospira* spp. through eating infected rodents or coming into contact with a contaminated environment (Brasil de Lima et al., 2014). As a result, cats may become infected with Leptospira spp. and play a role in disease transmission to other animals and humans. Despite epidemiologic studies have connected interactions with cats to an increased risk of human leptospirosis, there are no publications about leptospirosis in domestic cats in Chiang Mai. Therefore, the knowledge of this study about Leptospiral infection in cat in specific places is critical to understanding leptospirosis epidemiology in order to provide information on the development of efficient disease control and future strategies to eradicate this disease.

Materials and Methods

Animals and ethics

During 2017-2018, cats were treated at a feline medical clinic at Small Animal Hospital, Faculty of Veterinary Medicine, Chiang Mai University. There are no restrictions on age or breed. All cats participating in the program have consent from their owners and have undergone ethical considerations for animal use. This study was approved by the Ethics Committee for

Scientific Laboratory Animals Use, Faculty of Veterinary Medicine, Chiang Mai University with No. R1/2256.

History taking and clinical examination

In all cases, samples were collected following a check-up and clinical information such as clinical signs and vaccination programs were recorded by a veterinarian. 2 mL of whole blood was collected from the cephalic vein using a 23-gauge needle and placed in plain tubes for further investigation and collection of serum to detect hematological and biochemical values, which were centrifuged at 4000 rpm for 5 min for serological study. For future experiments, serum samples were stored at -20°C. All remaining specimens and extractions were kept at -80°C for future experiments. Urine samples were obtained using an ultrasound-guided cystocentesis and transferred into sterile tubes before being stored at 4 °C for real-time PCR techniques.

Serology

Serum samples were diluted to two-fold serial dilution (1: 50 - 1: 3200). Add diluted serum samples of 7 microliters per hole on slides of 1 to 7 respectively and prepare on 2 rows for IgG and IgM tests. Place the slides in a warm humidifier box at 37 ° C for 30 min. Rinse the slides with PBS 3 times for 5 min each time. After the slides dry, drop the Conjugated anti-human IgG-FITC and anti-human IgM-FITC 7 microliters per hole into rows 1 and 2, respectively. Place the slides in a warm humidifier box at 37 ° C for 30 min. Rinse the slides with PBS 3 times for 5 min at a time and then dry the slides with cool air. Mount slide with Glycerol buffer pH 8.5-9.0. Covered by a 24 x 60 mm cover slide. All slides were taken with a camera fluorescent microscope. Always test positive control serum and negative control serum with the test sample. The negative results were not found in Leptospira fluorescence. The positive results were green Leptospira fluorescence under camera fluorescent microscope.

Real-time PCR

The supernatants were discarded after centrifuging the serum samples (14,000 x g, room temperature) for 15 min. Phosphate buffered saline (PBS) was used to wash the pellets before placing them in 1.5 mL tubes. For the urine, the remaining sample was centrifuged (14,000 x g, room temperature) for 10 min, the pellet was rinsed with 500 µl phosphate-buffered saline and placed into 1.5 mL tubes after the supernatants were discarded. The supernatant was discarded after a second centrifugation, and the pellet was resuspended in 200 µl PBS. Total DNA was extracted by using NucleoSpin Tissue, Mini kit for DNA from cells and tissue, as directed by the manufacturer (Macherey-Nagel GmbH & Co., Germany). The quantity and quality of RNA were determined using a DU 730 Life Science UV/Vis spectrophotometry (Beckman Coulter Inc., USA).

For Leptospira RT-PCR was carried out using 7300 real-time RT-PCR systems (Applied Biosystems, USA) with Thunderbird™ SYBR® qPCR mix (Toyobo, Japan). The RT-PCR was

performed using primers and a probe that targeted the *lipL32* gene according to Bourhy et al., 2011 and Podgoršek et al., 2020, detecting only pathogenic Leptospira. ABI 7300 software was used to evaluate the data.

Statistical analysis

In this study, descriptive Statistics analyzed data from cats with Leptospirosis infection and normal cats without Leptospirosis infection with the data presented as a percentage.

Results and Discussion

All total of 80 mature cats were collected from Small Animal Hospital, Faculty of Veterinary Medicine, Chiang Mai University. 48 were female (60%) and 26 were male (32.5%). 5 were Neutered female (6.25%) and only one was neutered male cat (1.25%). Despite the fact that 72 cats were raised outside the home (90%). Most cats were raised outside home. In this study, 40 cats were recruited with evidence of clinical signs such as 8.75% anorexia, 7.5% nasal discharge, 2.5% depression, oliguria, vomit and 1.25% anemia, diarrhea, icterus, rhinitis and ilium fracture, respectively. Using test kits for viral infection diagnosis, 3 cats were positive FIV (3.75%). 2 cats were FPV (2.5%). One cat was positive FcoV (1.25%) (shown on Table 1). For detection of leptospira by IFA test, there were no positive results in all serum samples. When compared to the positive control group, which had a green glow under fluorescence microscopy, the results of the microscopic investigation revealed that none of the serum samples had a green glow under fluorescence microscopy (Figure 1).

Otherwise, two cats were positive using RT-PCR. The first cat was RT-PCR positive (1.25%) for blood sample. Urine sample of two cats were RT-PCR positive (2.5%) which one cat shown blood and urine RT-PCR positive on Table 2. One of the two urine RT-PCR positive cats was 5 years old, intact, female domestic short hair. The cat lived in Doi Tao sub-district, Doi Tao, Chiang Mai. The cat was privately owned, drank out of puddles, and potentially had contact to rodents. This cat had no history of vaccination that was presented FeLV Ag positive. The second cat was 3 years old, intact, male DSH which found blood and urine RT-PCR positive. This cat was raised in Faham sub-district, Muang, Chiang Mai with 3 cats on outdoor that all cats had a history of vaccination. The patient shown icteric mucous membrane, depression, anorexia, vomit, and dehydration about 7%.

Recently, serosurveys pronounced seropositive in cats for Leptospirosis in various geographic regions (Gamage et al., 2011; Sprißler et al., 2019). In current serosurveys, it was revealed that in various geographic regions, between 4.8 percent to 35.3 percent of cats tested were seropositive for Leptospira spp. (Millán et al., 2009; Markovich et al., 2012; Mylonakis et al., 2005; Sprißler et al., 2019). In Canada, *Leptospira* spp. was found infection in cats on eastern Canadian veterinary teaching hospital. Using microscopic agglutination test,

Table 1 The information of domestic cats was tested in Small Animal Hospital, Chiang Mai University (N = 80)

Gender (%)	Age (%)	Environment (%)	Vaccination (%)	Clinical signs (%)
Male = 26 (32.50) Neutered male = 1 (1.25)	< 4 yrs = 56 (70.00)	Indoor = 8 (10.00)	- FPV, FeLV, RV = 7 (8.75) - FPV, RV = 3 (3.75)	- Anorexia = 7 (8.75) - Anemia = 1 (1.25) - None = 20 (25)
Female = 48 (60.00) Neutered female = 5 (6.25)	≥ 4 yrs = 24 (30.00)	Outdoor = 72 (90.00)	- RV = 6 (6.25) - FcoV = 1 (1.25) - FIV = 3 (3.75) - FPV = 2 (2.50) - None = 65 (81.25)	- Depression = 2 (2.50) - Diarrhea = 1 (1.25) - Icterus = 7 (8.75) - Ilium fracture = 1 (1.25) - Nasal discharge = 6 (7.50) - Oliguria = 2 (2.50) - Rhinitis = 1 (1.25) - Vomit = 2 (2.50) - None = 40 (50)

FPV: Feline Panleukemia vaccine; FeLV: Feline Leukemia vaccine; RV: Rabies vaccine; FcoV: Feline coronavirus; FIV: Feline immunodeficiency virus.

they discovered that 10 of 40 cats tested positive for Bratislava, with two of them also testing positive for Autumnalis (Lapointe et al., 2013).

In 2019, Sprißler et al. investigated at whether outdoor cats in Thailand shed pathogenic Leptospira spp. in their urine, as well as the prevalence of antibodies and risk factors linked to Leptospiral infection. The result showed that real time PCR targeting the lipL32 gene of pathogenic Leptospira positive urine samples were found in 2/260 cats, but none of the 260 urine samples were culture positive. Additionally, antibodies to Leptospira were found in 14 of 260 cats, with titers ranging from 1:20 to 1:160. This study demonstrated that outdoor cats can be a source of infection for humans in Thailand because they can excrete DNA as well as potentially viable Leptospira in their urine. In a more recent study, 29.3% (n = 24/ 82) of healthy cats from two shelters from Malaysia were positive by serological, molecular, and/or culture methods. Interestingly, through serological and molecular approaches, the pathogenic *Leptospira* spp. isolates were identified as *L. interrogans* serovar Bataviae. (Alashraf et al., 2020). In another study in southern Chile, a total 231 outdoor cats were

Table 2 Results of Hematology, Blood chemistry and DNA detection in blood and urine shedding by RT-PCR in cats from Small Animal Hospital, Chiang Mai University

Gender (Age)	Environment	Clinical signs	Hematology & Blood chemistry profile	Positive result by RT-PCR
Male (3 years)	outdoor	Anorexia, Vomit, Icterus	Azotemia, Hyperglobulinemia, Hyperbilirubinemia, Leukocytosis, Thrombocytosis, Hyponatremia, Hypochloremia,	Urine and serum
Female (5 years)	outdoor	Anorexia, Weakness, Depression	Severe anemia, Leukocytosis, neutrophilia with left shift, Eosinophilia, Monocytosis Severe Thrombocytopenia, Increase ALT& AST, Hypobilirubinemia, Hyperproteinemia, Hyperglobulinemia, Increase BUN, Hypercalcemia, Hyperkalemia	Urine

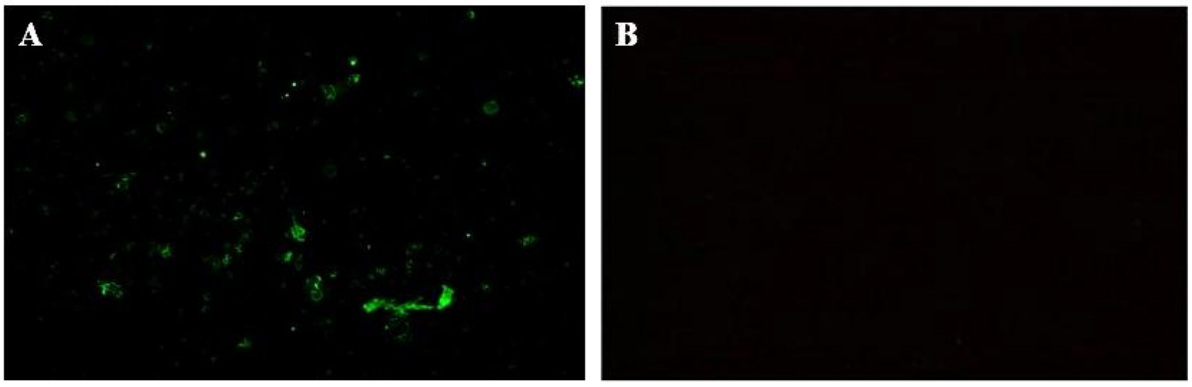

Figure 1 The results from serum samples under fluorescence microscopy by immunofluorescence assay (IFA); A = the positive control glow green, B = every field was dark in color, with no green glow.

sampling from both rural and urban regions. The findings revealed that thirty-six urine samples tested positive for Leptospira using both molecular and conventional culture approaches,

with qPCR yielding a higher positive rate than conventional culture. (Dorsch et al., 2020). This suggests that using qPCR to detect leptospiral DNA in feline urine improves sensitivity considerably. Taken together, the positive result by real time PCR of Leptospirosis in cat was shown 2.5% (2/80 cats) in our Chiang Mai University Small Animal Veterinary Teaching Hospital from Muang and Doi Tao areas can be a possible source of infection for people.

In this investigation, two adult outdoor cats tested positive for Leptospirosis. By hunting and eating, the cat had a significant risk of coming into contact with infected rodents or contaminated source (Chan et al., 2014). Leptospiral infection in cats was caused either directly by swallowing contaminated prey such as rats or indirectly by drinking polluted water from domestic animals, rodents, and wildlife (Green et al., 2011). For clinical examination, Anorexia, depression, weakness, vomiting, and icterus were all observed in the infected two cats. In previous report, the clinical signs of leptospira infection were observed polyuria, polydipsia, uveitis, and lameness in cats. They stated that clinical signs may appear after a long peroid of infection (Arbour et al., 2012). Moreover, an older cat was identified as a risk factor in many publications. (Larsson et al.,1984; Brasil de Lima et al., 2014; Mosallanejad et al., 2011).

In this research, leptospiral DNA was found in the urine of infected cats, but IFA testing was negative. The cats were most likely infected very early, the infecting serovar was not included in the IFA panel, or antibodies had fallen to levels below the IFA's detection limit by the time the sample was obtained. The short period for data collection, the narrow geographic area, and the small number of cats are all limitations of this study. Furthermore, the lack of information on the carrier status of positive cats, as well as the clinical disease of infected cats, require further examination.

Conclusions

In conclusion, this is the first report of Leptospira spp. molecular and serological detection in domestic cats from Chiang Mai University Small Animal Veterinary Teaching Hospital, Chiang Mai, Thailand. By using RT-PCR, we found that Leptospiral infection is present in cats in our hospital population. As a result, cats can infect hospital personnel such as veterinarians and other medical personnel. More research is needed to determine the function of cats as a source of infection in both animals and people, as well as the potential of zoonotic transmission.

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