Jurnal Veterinar Malaysia

ISSN 0128-2506

Vol. *32* No. *1* (July) *2020*

Veterinary Association Malaysia

J. Vet. Malaysia (2020) 32 (1): 1-4

Journal Articles

OCCURRENCE OF *Corynebacterium ulcerans* AND *Pasteurella multocida* IN PET CATS AND DOGS IN KLANG VALLEY, MALAYSIA

N.G. LIM, A.A SALEHA, G.K. DHALIWAL, S. KHAIRANI-BEJO and K.H. KHOR

Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

SUMMARY

Pets especially cats and dogs are known to carry a number of zoonotic pathogens in their mouths, such as *Pasteurella multocida* and *Staphylococcus* species while *Corynebacterium ulcerans* is reported to be emerging in these animals. *C. ulcerans* produces diphtheria toxin and causes diphtheria-like symptoms in human. This infection is an emerging public health threat in developed countries, with incidence sometimes higher than that of *C. diphtheriae.* Infection due to *P. multocida* is often associated with bite wounds and scratches that cause significant morbidity and mortality in humans. Therefore, this study was conducted to determine the occurrence of *C. ulcerans* and *P. multocida* in pet cats and dogs in Klang Valley, Malaysia and their antibiotic resistance profiles. Nasal and pharyngeal samples were collected from apparently healthy animals comprising 26 cats and 29 dogs that were presented at four veterinary health care facilities in Klang Valley. The isolates were tested against six antibiotics commonly used in small animal practice. *C. ulcerans* was not isolated (0%) in this study whereas the occurrence for *P. multocida* was low (10.9%). *P. multocida* isolates showed low resistance (16.7% each) to amoxicillin-clavulanic acid, tetracycline, trimethoprim-sulfamethoxazole and cephalexin.

Keywords: *Corynebacterium ulcerans*, *Pasteurella multocida*, cats, dogs

INTRODUCTION

Pet animals including cats and dogs carry a number of zoonotic bacterial pathogens which pets can transmit to humans. One common bacterial species reported is *Pasteurella multocida* while *Corynebacterium ulcerans* is reported as emerging.

Some strains of *C. ulcerans* produce diphtheria toxin (DT) which causes diphtheria-like diseases in humans (Saeki *et al.*, 2015), is similar to the one caused by *Corynebacterium diphtheriae*. *C. ulcerans* infection in humans was previously associated with consumption of raw milk and dairy products or in closed contact with cattle and goats. Besides the farm animals, *C. ulcerans* diphtheria has been increasingly reported and possible transmission from pet cats or dogs has been speculated. In pet animals, *C. ulcerans* has been isolated from both healthy asymptomatic carriers and clinically affected animals with upper respiratory signs, bronchopneumonia, and/or skin lesions. In a review paper by de Oliveira Dias et. al. (2011), it was reported that there were more documented cases of *C. ulcerans* infections in cats and dogs compared to other animals.

P. multocida has been isolated as part of the normal oral and pharyngeal flora of many animals, including cats and dogs. *P. multocida* infection in humans can manifest as pneumonia, atrophic rhinitis, dermonecrosis, cellulitis, abscesses, meningitis, and/or haemorrhagic septicaemia, which may lead to significant morbidity and mortality (Wilson and Ho, 2013). Transmission of human infection were often due to post incidence of bites and scratches form the close contact between the pets and their owners (Chomel and Sun, 2011).

Therefore, the objectives of this study were to determine the occurrence of *C. ulcerans* and *P. multocida* in pet cats and dogs in Klang Valley, Malaysia and their antibiotic resistance profiles. Information obtained will allow a better understanding of the occurrences of the disease locally and the suitable treatment regime that can be proposed.

MATERIALS AND METHODS

Specimens collection

Nasal and pharyngeal swabs were collected from 55 apparently healthy animals consisted of 26 cats and 29 dogs. These animals were sampled at four veterinary health care facilities in Klang Valley, Malaysia. For isolation of *C. ulcerans*, samples from the nasal and pharyngeal area were taken from each animal using a sterile swab that were immediately placed in modified Stuart transport media (Oxoid, UK). For isolation of *P. multocida*, another pharyngeal swab was taken and placed in sterile Cary Blair transport media (Labchem, UK) for isolation of *P. multocida* (Kawamoto et al., 1997). All the swabs were in a cool box with ice packs and transferred to the Veterinary Public Health Laboratory at Faculty of Veterinary Medicine.

Corresponding author: Dr Saleha Abdul Aziz (A.A. Saleha);
Email: saleha@upm.edu.my

Editorial history
Paper received: 2nd April 2020
Accepted for publication: 21st May 2020
Issue Online: July 2020

Isolation and Identification of Corynebacterium ulcerans

Each nasal and pharyngeal swab specimen was streaked on horse blood agar (Oxoid, UK) and Hoyle tellurite blood agar (HTBA) (Oxoid, UK) (Public Health England, 2014) for isolation of *C. ulcerans*. The blood agar plates were then incubated in candle jar for 24 hours at 37°C and the HTBA plates were incubated aerobically for 24 - 48 hours at 37°C. On horse blood agar, the *C. ulcerans* colonies were described as grey or white, dry, with waxy consistency, circular, slightly convex with an entire margin and exhibit a small zone of β-hemolysis whereas on HTBA plates, the grey or black, very dry opaque colonies were suspected as *C. ulcerans* (Berger *et al*., 2013). Gram staining was carried out on all suspected colonies and those showed Gram positive bacilli were then subcultured. Biochemical tests were then performed. Organisms positive for catalase, urease, hemolysis and trehalose tests and negative for nitrate and sucrose tests were presumptively identified as *C. ulcerans*. (Jang *et al*., 2008). Further identification were conducted using the rapid identification kit, RapID CB Plus System (Remel, USA). Remel RapIDTM CB Plus System is a qualitative micromethod employing conventional and chromogenic substrates for the identification of medically important coryneform bacteria (23 species including *C. ulcerans*), listeriae (6 species) and other irregular gram-positive bacilli (19 species); each panel consisted of 18 reactions and colour changes were noted after incubation at 35ºC for 4 hours aerobically.

Isolation and Identification of Pasteurella multocida

Each pharyngeal swab specimen was streaked on horse blood agar and incubated aerobically at 37°C for 24 hours for isolation of *P. multocida*. Colonies that were convex, smooth, greyish and non-hemolytic were suspected that of *P. multocida* (Tille, 2014). Gram staining was also carried out on all suspected colonies and those showed Gram negative coccobacilli were subcultured. Organisms positive for oxidase and indole tests and negative for urease, motility, hemolysis, ONPG tests and growth on MacConkey agar were presumptively identified to be *P. multocida* (Jang *et al*., 2008). Similar as *C. ulcerans*, the rapid identification kit, RapID NF Plus System (Remel, USA) was used for identification of medically important glucose-nonfermenting, gram-negative bacteria and other select glucose-fermenting, gram-negative bacteria not belonging to the family *Enterobacteriaceae* with the differential chart consisted of a total of 73 species including *P. multocida*. Colour changes were observed on17 different reactions which were noted after incubation at 35ºC for 4 hours aerobically.

Antibiotic Susceptibility Test (AST)

Antibiotic susceptibility tests were performed using the Kirby-Bauer disc diffusion method. In brief, each isolate was suspended in sterile saline at a concentration of 0.5 MacFarland. The bacterial suspension was then spread with a cotton swab over Mueller-Hinton agar (Oxoid, UK) supplemented with 5% horse blood. Six antibiotic discs were used, namely amoxicillin-clavulanic acid (30µg), tetracycline (30µg), enrofloxacin (5µg), trimethoprim-sulfamethoxazole (25µg), cephalexin (30µ) and erythromycin (15µg) were placed on the agar surface using an antibiotic disc dispenser. All plates were incubated at 37ºC for 24 hours. Zones of inhibitions around antibiotic discs, if present, were measured using a caliper.

RESULTS

Occurrence of Corynebacterium ulcerans

From this study, *C. ulcerans* (0%) was not isolated, however other *Corynebacteria* species were identified using the RapID CB Plus System, namely *C. striatum*., *C. jeikeium* and *C. afermentans* ss *afermentans* (42.3% in cats and 82.8% in dogs). Besides that, *Brevibacterium casei* (26.7%), *Leifsonia aquatica* and *Oerskovia* spp. (6.7% each) were identified.

Occurrence of Pasteurella multocida

The occurrence of *P. multocida* was 10.9% (6 out of 55 animals). The occurrence in cats was higher at 15.4% whereas in dogs it was 6.9%. Other *Pasteurella* species that were also isolated from this study included *P. canis* and *P. aerogenes* (only in dogs at 20.7% and 3.4%, respectively), *P. pneumotropica* (only in cats at 15.4%), *P. stomatis* (in dogs at 6.9% and in cats 3.8%). Other Gram-negative bacilli isolated were *Moraxella* spp. (43.6%), *Moraxella canis* (3.6%), *Proteus mirabilis* (7.3%), *Actinobacillus ureae* (9.1%), *Klebsiella pneumoniae* 1.8%), *Aggregatibacter actinomycetemcomitans* (10.9%) and *Acinetobacter baumanii* (1.8%).

Antibiotic Susceptibility Test (AST)

P. multocida isolates showed low resistance to amoxicillin-clavulanic acid, tetracycline, trimethoprim-sulfamethoxazole and cephalexin (16.7% each). Only one isolate had intermediate susceptibility to enrofloxacin whereas 3 or 50% of the isolates had intermediate susceptibility to erythromycin. Only one isolate showed resistance to antibiotics, that is, resistant to four of six antibiotics tested and was susceptible to enrofloxacin and erythromycin.

DISCUSSION

C. ulcerans was not isolated in this study which might suggested the absence or a very low presence of this bacteria among the pet cats and dogs in Klang Valley. However, results must be interpreted with limitation of small sample size, the usage of blood agar and that HTBA was more suitable for isolation of *C. diphtheriae*. According to Katsukawa et. al., (2009), the Katsukawa medium would be more suitable for use as it was more selective of *C. ulcerans* compared to the blood agar that were used; however this medium was not

readily available here. Besides that, Katsukawa medium was also reported as more suitable for the selection of *C. ulcerans* from specimens that contained small numbers of the organism.

As mentioned earlier, cats and dogs are increasingly recognised as important sources of *C. ulcerans* infection in humans. A number of quite recent reports had documented cases of C. *ulcerans* in humans acquired from cats and dogs. According to Saeki et. al. (2015), twelve cases of diphtheria or diphtheria-like disease associated with toxigenic *C. ulcerans* have been reported in Japan since toxigenic *C. ulcerans* was first detected in 2001. In these several cases, patients were confirmed as having had direct contact with dogs or cats with/without dermatitis or respiratory symptoms such as rhinitis. Otsuji et. al. (2017) reported the first fatal case of *C. ulcerans* in Japan where the woman had contracted the infection from her pet cat. In another report by Berger et. al. (2011), a woman in Germany with pharyngeal diphtheria-like illness had acquired her infection also from her asymptomatic pet cat while Meinel et. al. (2015) reported in a man diagnosed severe necrotizing fasciitis in the calves of both legs caused by toxigenic *C. ulcerans* and one of his two pet dogs was found to harbour similar toxigenic *C. ulcerans.* Meinel et al (2015) also mentioned reports on the isolations of undistinguishable toxigenic *C. ulceran* strains from two dogs and two cats and their respective owners, too.

Generally, *C. ulcerans* is susceptible to most antibiotics such as penicillin, amoxicillin, tetracycline, ceftriaxone and erythromycin (Berger, 2011, 2012; Hirai-Yuki, 2013, Meinel, 2015). *C. ulcerans* infections in cats and dogs can be difficult to treat as reported by several literatures. The reduced effectiveness of the drugs may be due to metabolic factors within the host, inability of the drugs to access the infection site, inefficient transport into the bacteria, or the host may be infected repeatedly (Katsukawa, 2012). Since many domestic and wild animals may serve as reservoirs for *C. ulcerans*, one possible preventive measure is by developing an effective vaccine to prevent animals from infection (Katsukawa, 2012) which can directly prevent transmission to humans.

The occurrence of *P. multocida* among pet cats and dogs in Klang Valley was 10.9% in this study. In cats, the occurrence was 15.4%, whereas in dogs the occurrence was 6.9%. This suggested that *P. multocida* would cause wound infection more often in cases of cat bites than dog bites wounds in human. Apart from *P. multocida*, other species of *Pasteurella* were also isolated. In United States, it was reported that *Pasteurella* species, in particular *P. multocida*, were isolated from infections resulting from 50% of dog bites and 75% of cat bites; apart from bites and scratches, infection with *P. multocida* can occur as a results of kissing, licking of skin abrasions and mucosal surfaces (eyes, nose and mouth) in contact with mucous secretions from pets (Wilson and Ho, 2013). Wilson and Ho (2013) also reported that "over the past 30 years saw 20-30 human deaths due to pasteurellosis occur annually worldwide and that this rate appears to be increasing"; in all cases, deaths appeared to be due to complications with *P. multocida*. Isolation of *P. canis* was reported more prevalent with dog bites.

In general, *P. multocida* is susceptible to most β-lactams and β-lactamases inhibitor (clavulanic acid), which often make these the best treatment of choice by many medical practitioners (Mitnovetski & Kimble, 2004; Naba *et al*., 2009; Yamaguchi *et al*., 2014) in treating wounds.

The severity of *P. multocida* infections can be minimised by early aggressive antibiotic therapy prior to availability of microbiology results. As *P. multocida* is considered a microflora of the oral cavity of cats and dogs, efforts to eliminate the bacteria may seem futile. A possible preventive measure is by developing vaccines to prevent infection in humans. In the meantime, individuals such as pet owners can improve their personal hygiene and avoid kissing or sleeping with their pets. Every incidence of bite wound obtained by owners from their pets must be treated and managed accordingly.

CONCLUSION

The study showed the absence of *C. ulcerans* and low occurrence of *P. multocida* (10.9%) in pet cats and dogs in Klang Valley, Malaysia. For *P. multocida*, low antibiotic resistance were observed for amoxicillin-clavulanic acid, tetracycline, trimethoprime-sulfamethoxazole and cephalexin (16.7% each). Fifty percent (50%) of the isolates had intermediate susceptibility to erythromycin. *C. ulcerans* is reported to be circulating in many countries and is becoming an emerging public health threat. Future studies should use a larger sampling size and include stray animals to better reflect the current situation in Malaysia. Identification techniques which have a higher sensitivity such as PCR can also be used. Both *C. ulcerans* and *P. multocida* are zoonotic agents that can cause severe diseases in humans. Therefore, it is necessary to raise awareness among pet owners regarding the zoonotic risks of *C. ulcerans* and *P. multocida* that their pets may carry.

CONFLICT OF INTEREST

None of the authors have any potential conflicts of interest to declare.

ACKNOWLEDGEMENT

We would like to thank staff of the veterinary health care facilities and the pet owners for their support and participation and technical staff of Veterinary Public Health and Bacteriology Laboratories for their kind assistance.

REFERENCES

Berger, A., Huber, I., Merbecks, S.S., Ehrhard, I., Konrad, R., Hörmansdorfer, S., Hogardt, M. & Sing, A. (2011). Toxigenic *Corynebacterium ulcerans* in woman and cat. Emerging Infectious Disease Journal. 17(9): 1767–1769.

Berger, A., Trcek, J., Hogardt, M., Hörmansdorfer, S., Rau, J. & Sing, A. (2012). Antimicrobial resistance of *Corynebacterium ulcerans* and *C. pseudotuberculosis*. [Abstract]. Antimicrobial susceptibility testing - EUCAST and beyond. Retrieved February

9, 2015 from http://registration.akm.ch/einsicht.php?XNABSTRACT_ID=140203&XNSPRACHE_ID=2&XNKONGRESS_ID=161&XNMASKEN_ID=900

Berger, A., Hogardt, M., Konrad, R., & Sing, A. (2013). Detection methods for laboratory diagnosis of diphtheria. In A. Burkovski (Ed.), *Corynebacterium diphtheriae* and Related Toxigenic Species: Genomics, Pathogenicity and Applications (p. 177). Dordrecht: Springer.

Chomel, B.B. & Sun, B. (2011). Zoonoses in the Bedroom. Emerging Infectious Disease Journal. 17(2): 167-172

de Oliveira Dias, A.A.d.S, Santos, L.S., Sabbadini, P.S., Santos, C.S., Silva Junior, F.C., Napoleão, F., Nagao, P.E., VillasBôas, M.H.S., Hirata Junior, R. & Guarald, A.L. M. (2011) . *Corynebacterium ulcerans* diphtheria: an emerging zoonosis in Brazil and worldwide. Revista de Saúde Pública. 45(6): 1-16.

Hirai-Yuki, A., Komiya, T., Suzaki, Y., Ami, Y., Katsukawa, C., Takahashi, M., Yamamoto, A. Yamada, Y.K. (2013). Akihiko Yamamoto, Yasuko K YamadaIsolation and characterization of toxigenic *Corynebacterium ulcerans* from 2 closed colonies of cynomolgus macaques (*Macaca fascicularis*) in Japan. Comparative Medicine. 63: 272–278.

Jang, S.S., Biberstein, E.L. & Hirsh, D.C. (2008). A Diagnostic Manual of Veterinary Clinical Bacteriology and Mycology.

Katsukawa, C., Kawahara, R., Inoue, K., Ishii, A., Yamagishi, H., Kida, K., Nishino, S., Nagahama, S., Komiya, T., Iwaki, M., & Takahashi, M. (2009). Toxigenic Corynebacterium ulcerans isolated from the domestic dog for the first time in Japan. Japanese Journal of Infectious Disease. 62, 171-172

Katsukawa, C., Komiya, T., Yamagishi, H., Ishii, A., Nishino, S., Nagahama, S., Iwaki, M., Yamamoto, A., & Takahashi, M. (2012). Prevalence of Corynebacterium ulcerans in dogs in Osaka, Japan. Journal of Medical Mmicrobiology, 61(Pt 2): 266–273. https://doi.org/10.1099/jmm.0.034868-0

Meinel, D. M., Konrad, R., Berger, A., König, C., Schmidt-Wieland, T., Hogardt, M., Bischoff, H., Ackermann, N., Hörmansdorfer, S., Krebs, S., Blum, H., Margos, G., & Sing, A. (2015). Zoonotic transmission of toxigenic Corynebacterium ulcerans strain, Germany, 2012. Emerging infectious diseases, 21(2), 356–358. https://doi.org/10.3201/eid2102.141160

Mitnovetski, S. & Kimble, F. (2004). Cat bites of the hand. ANZ Journal of Surgery, 74: 859–862.

Naba, M.R., Araj, G.F., Kanafani, Z.A. & Kanj, S.S. (2008). First case of *Pasteurella multocida* endocarditis of the tricuspid valve: a favorable outcome following medical treatment. International Journal of Infectious Disease. 13(5):e267-269.

Otsuji, K., Fukuda, K., Endo, T., Shimizu, S., Harayama, N., Ogawa, M., Yamamoto, A., Umeda, K., Umata, T., Seki, H., Iwaki, M., Kamochi, M., & Saito, M. (2017). The first fatal case of Corynebacterium ulcerans infection in Japan. JMM case reports, 4(8), e005106. https://doi.org/10.1099/jmmcr.0.005106

Public Health England. (2014). UK Standards for Microbiology Investigations: Identification of *Corynebacterium* species. Retrieved February 8, 2015 from www.gov.uk/government/uploads/system/uploads/attachment_data/file/371490/ID_2i4.1.pdf

Saeki, J., Katsukawa, C., Matsubayashi, M., Nakanishi, H., Furuya, M., Tani, H. & Sasai, K. (2015). The detection of toxigenic *Corynebacterium ulcerans* from cats with nasal inflammation in Japan. Epidemiology and Infection. 143: 2660–2665.

Tille, P. M. (2014). *Pasteurella* and similar organisms. In Bailey & Scotts Diagnostic Microbiology (13th ed., pp. 393). St. Louis, MO: Elsevier Mosby.

Wilson, B.A. & Ho, M. (2013). *Pasteurella multocida*: from zoonosis to cellular microbiology. Clinical Microbiology Review. 26(3):631–655.

Yamaguchi, H., Tamura, T., Abe, M., Ogiwara, S., Sai, S., Kosugiyama, K., Sugihara, A., Nagumo, K., Iwata, S., & Kinugawa, Y. (2014). Prolonged incubation period in neonatal Pasteurella multocida meningitis and bacteremia. Pediatrics international official journal of the Japan Pediatric Society, 56(6), e79–e81. https://doi.org/10.1111/ped.12432