

## Detection of protozoan and bacterial pathogens of public health importance in faeces of *Corvus* spp. (large-billed crow)

Lee Hwee Yong, Stephen Ambu, Sushela Devi and Mala Maung

International Medical University, 126, Jalan 19/155B, Bukit Jalil, 57000 Kuala Lumpur, Malaysia.

Email corresponding author: Stephen\_ambu@imu.edu.my

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**Abstract.** Parasites and bacteria are reported in the faeces of birds in the current study. Fresh faecal samples of the large-billed crow (*Corvus* spp.) were collected from the study site at Bangsar, an urban setting in Kuala Lumpur, Malaysia. These samples were transported to laboratory and analysed for parasites and bacteria. Pre-prepared XLD agar plates were used for culturing the bacteria in the laboratory. Using the API 20E™ Test Strips, 9 different species of bacteria were identified belonging to the family Enterobacteriaceae. They were *Citrobacter freundii*, *Enterobacter cloacae*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Kluyvera ascorbata*, *Salmonella arizonae*, *Salmonella typhi*, *Shigella flexneri* and *Shigella sonnei*. The protozoan parasites detected include *Cryptosporidium* spp., *Cyclospora* spp., *Blastocystis* spp., and *Capillaria hepatica* and *Ascaris lumbricoides* ova. Environmental air samples collected on agar plates using an air sampler in the area only produced fungal colonies. Some of these pathogens found in the crows are of zoonotic importance, especially *Cryptosporidium*, *Blastocystis*, *Cyclospora*, *Salmonella*, *Shigella* and *Kluyvera*. The finding of *Kluyvera* spp. in crows in our current study highlights its zoonotic potential in an urban setting.

### INTRODUCTION

The emergence of diseases such as the Nipah Virus, Severe Acute Respiratory Syndrome and the Avian Influenza has made Asians more conscious of the role of wild life in the transmission of pathogens of human health importance. Chua *et al.* (1999) have reported on the role of animals such as pigs and horses in the transmission of the Nipah and Hendra viruses. Graczyk *et al.* (1998) and Rohela *et al.* (2005) identified wild and domestic animals as a source of transmission of *Cryptosporidium* spp. There are also reports that indicate increased levels of bacteria in public water supplies and source of contamination attributed to some species of wild birds (Bangert *et al.* 1988; Hopkins *et al.*, 1990; Feare *et al.*, 1999). Studies show that *Cryptosporidium* spp. can be transmitted through the faecal-oral route,

due to consuming contaminated food or water, or swimming in contaminated waters (Cheesebrough, 1987). One good example is the Milwaukee outbreak of 1993 in the United States of America where 400,000 people were infected when the public water supplies were contaminated (Fayer, 2000). *Cyclospora* spp. which is a common cause of traveler's diarrhea has caused public health concern in many developing countries (Cheesebrough, 1987) and therefore is an important parasite to monitor. However the pathogenicity of *Blastocystis* spp. is still controversial (Leelayoova *et al.*, 2001), and its importance as a public health concern is difficult to assess but nevertheless it cannot be underestimated as it has a high infection rate amongst primates, pheasants and ducks (Abe *et al.*, 2002).

Nematodes such as *Capillaria* spp. and *Ascaris* spp. are usually seen in birds as a

consequence of the bird having eaten another animal that has been infected with these parasites (Cheesebrough, 1987). Fish eating birds are now considered natural definitive host of *Capillaria hepatica*. Therefore it is important that wild birds, especially scavengers such as the crow be considered as potential sources for the contamination of water supplies with ova of these two parasites.

The importance of bacteria and their related health importance are reported in many studies. Gram negative bacteria such as *Enterobacter* spp., *Klebsiella* spp., *Salmonella* spp., *Shigella* spp. and *Proteus* spp. (Bangert *et al.*, 1988, Flemer & Drewes, 1988) which have important public health concerns have been detected in bird faeces. In a study on large flocks of water fowls by Hussong *et al.* (1979) it was found that these birds contributed to a substantial increase in the densities of faecal coliforms in the surrounding water sources. In another study, Sarria *et al.* (2001) found *Kluyvera* spp. in bird faeces. This bacteria was considered to have no health consequence but is now known to cause infrequent infections in humans.

Crows were chosen as the study subject mainly because they are known to roost very well in urban areas, adapting well to the competitive environment by scavenging for food (Dickinson *et al.*, 2004). The house crow is a public health hazard and can be a threat to tourist amenities as studies have shown that crows carry *Salmonella*, *Plesiomonas*, enteropathogenic *Escherichia coli*, *Shigella* and *Aeromonas hydrophila* (Meier, 2007). Ngui (2004) has reported that crow and rats have become a potential health hazard due to their large numbers attributed to the extensive commercial activities in the Bangsar area.

The study site, opposite the TMC Minimarket in Bangsar is well known for its crow problems, and it is a residential cum commercial hub of the city with very active people movement during day and night. The aim of the study was to analyse the crow faeces collected from this area to create a checklist of parasites and bacteria of human health importance and to establish the role

and importance of birds in their transmission.

## MATERIALS AND METHODS

Faecal droppings from crows were collected at around 7 pm in the evening, as by this time the crows return to the roosting position in the trees surrounding the TMC minimarket at Bangsar, Kuala Lumpur. Only fresh faecal samples were collected from the ground immediately after they dropped from the crows' roosting position. The faeces was scooped off the ground using an applicator stick and put into screw-capped plastic centrifuge tubes. These sample tubes were pre-filled with selenite broth for bacterial samples and phosphate buffered saline (PBS) for bacteria and parasites (two tubes per sample).

In the laboratory, the samples (50 ml) were mixed with selenite broth (10 ml), shaken and incubated at 37°C for 24 hours. A 20 *ul* sample of this culture was used for streaking on the pre-prepared plates with XLD agar for bacterial growth. However for the detection of *Salmonella* sp. and *Shigella* sp., the selenite broth sample was incubated for a longer period of time (24-72 hours) for optimum bacterial growth and a 20 *ul* sample streaked onto the pre-prepared XLD agar plates.

A simple key was used to identify the various bacterial species. Red colonies indicated *Shigella* spp. or *Salmonella* spp.; pink colonies – *Proteus mirabilis*; yellow colonies – *Escherichia coli*, *Enterobacter* spp., *Aeromonas* spp., *Klebsiella* spp., *Citrobacter* spp., *Serratia* spp., *Hafnia* spp. and *Proteus vulgaris*.

The API 20E™ strips were used for confirming the bacteria species such as the Enterobacteriaceae and non-fastidious gram-negative rods. The reactions were read according to the Reading Table provided and the identification obtained by using the Analytical Profile Index.

Air samples (Biomerieux™ Air IDEAL Sampler) were collected (suction velocity at 100 litres/minute) on pre-prepared XLD agar plates at time intervals of 5, 10 and 15

minutes to determine the optimal collection time and thereafter all samples were collected at 10 minute intervals. The samples were processed as the bacterial samples above.

The samples in PBS for parasitological studies were thoroughly mixed and centrifuged at 2000 rpm for 1 minute and analysed using the formol ether concentration method. Ten *ul* of the concentrated sample was smeared on albumin coated slides, dried and stained using the Trichrome and Ziehl-Neelsen stains. The parasites were identified using a microscope.

## RESULTS

All 106 faecal cultures produced yellow colonies on XLD agar and 47 (44.3%) of these produced red colonies, indicating that *Shigella* or *Salmonella* were present in these samples. The API 20E™ Test Strips were used to identify the gram-negative bacteria in 50 randomly collected positive samples. Out of the 50 strips only 48 yielded positive results while the other two were not conclusive. A total of 9 different species of bacteria were identified belonging to the family Enterobacteriaceae. The bacteria and number of colonies present for each species was *Citrobacter freundii* (6), *Enterobacter cloacae* (9), *Proteus mirabilis* (9), *Klebsiella pneumoniae* (9), *Kluyvera ascorbata* (2), *Salmonella arizonae* (8), *Salmonella typhi* (4), *Shigella flexneri* (2) and *Shigella sonnei* (5).

For the 9 air samples taken at different time durations, the mean number of colonies

seen in the culture plates exposed for 5 minutes was very low (1.67) as compared to the 10 minute exposure samples (6) and the 15 minute exposure samples (4.33). The 10 minute exposure samples produced plates with the highest number of colonies, and this was taken as the optimum exposure time for the collection of air samples. The exposed culture plates (3) gave different numbers of colonies as shown in Table 1. Most of the colonies were found to be fungus, based on their morphology with the exception of one which was *Klebsiella* spp.

Table 1. Results of air samples taken on 3 different sampling days

Plate Number	Plate 1	Plate 2	Plate 3
Weather during collection	Dry	Damp	Dry
Total number of colonies	9	70	6
Mean number of colonies	3	23.33	2

Forty of the 106 samples were positive for parasites as shown in Table 2. Thirty one (29.24%), were found to have only one type of parasite, and the rest had 2 types.

Among the 31 samples that contained one type of parasite, 28 of them had only *Cryptosporidium* spp., 2 of them had *Capillaria hepatica* and 1 of them had only *Blastocystis* spp. Of the 10 samples which had two types of parasites, 6 of them contained both *Cryptosporidium* spp. and *Cyclospora* spp. All of the samples that had *Cyclospora* spp. had also *Cryptosporidium* spp. as well. Other than that, the samples that had two types of parasites, 3 of them contained *Cryptosporidium* sp. and

Table 2. Results of parasites in wet samples

Parasite	Number of samples +ve	Percentage (%)	Stain
<i>Cryptosporidium</i> spp.	36	33.96	Modified Ziehl-Neelsen/Trichrome
<i>Cyclospora</i> spp.	6	5.66	
<i>Capillaria hepatica</i>	3	2.83	
<i>Blastocystis</i> spp.	4	3.77	Trichrome
<i>Ascaris lumbricoides</i>	1	0.94	

*Blastocystis* sp. and 1 contained *C. hepatica* and *Ascaris lumbricoides*.

## DISCUSSION

A significant proportion of the faecal samples in our study were found to have *Cryptosporidium* spp., making it the most common protozoan parasite found. As *Cryptosporidium* spp., *Blastocystis* spp., *Cyclospora* sp., and *Capillaria hepatica* are spread through the faecal-oral route to humans (Cheesebrough, 1987), and that the crows, being scavengers with very versatile feeding habits (Dickinson, 2004), that will take food from the ground, or might have fed on items which have been contaminated by infectious droppings from another infected animal, such as rats or fishes (Angus, 1983) can become infected or be carriers of these pathogens and thus play a notable role in the transmission of these parasites.

Farizawati *et al.* (2005) have reported how animals can play a role in the transmission of human pathogens such as *Cryptosporidium* sp. and *Giardia* sp. through contamination of water sources such as Sungai Semenyih. Similarly, Rohela *et al.* (2005) found 6 species of exotic birds in the National Zoo at Kuala Lumpur infected with *Cryptosporidium* sp. These studies indicate that farm animals and wild birds can be potential sources of transmission of parasites in our urban and peri-urban areas. Crows are ubiquitous in distribution in Kuala Lumpur and the surrounding suburbs, therefore they can form a link in the transmission of these parasites from a contaminated water source or from other infected wild birds in the Zoo.

The list of bacteria that can be isolated from bird droppings is endless and some examples of bacteria in the floral gut of birds are given by Flemer & Drewes (1988). From our study, all of the samples tested positive for gram-negative Enterobacteria. *Salmonella* spp., *Shigella* spp., *Proteus* spp., *Citrobacter* spp., *Enterobacter* spp., *Klebsiella* spp., and *Kluyvera* spp. *Klebsiella* spp. was the only type of bacteria isolated from both faecal and air samples. *Kluyvera*

spp. has been detected more commonly in water, soil, sewage and cows but has also been occasionally isolated from clinical samples (Farmer *et al.*, 1981). This study identifies this bacteria to be an infrequent opportunistic pathogen which needs more information to establish its incidence and clinical significance. The finding of *Kluyvera* spp. in crows in our current study could be used as information for establishing its potential as a disease agent in an urban setting.

The air samples in our study produced mainly fungal colonies on cultures plate with the exception of one single *Klebsiella* spp. It was found that the number of colonies on the agar plates had some relationship to the prevailing environmental conditions under which the sample was collected. In this study, samples collected under damp conditions, after heavy rain, produced more colonies than samples collected during dry weather. Studies have shown sunlight, temperature and humidity to have an effect on bacteria, fungi, protozoans and viruses (Ignoffo, 1992). This study found UVB radiation from sunlight to be the most destructive factor for pathogens, while temperature in a normal environment did not have much effect. Similarly humidity or water did not have much effect on the survival of viral and bacterial pathogens but moisture reduced the infectivity of many protozoa. However, moisture was a primary requirement for germination of fungal spores. These attributes may be the reason why the samples collected during wet days in the current study yielded significantly higher number of colonies as compared to drier days.

In conclusion this study has highlighted some useful information that can be used for instituting crow control and environmental management in Bangsar which currently could be exposed to human pathogens from avian sources. The concern is the potential contamination of food and water in food stalls in this area by contact and air and its subsequent transmission to patrons. The crows roost in this area and their droppings are everywhere, thus contamination is inevitable. A well designed questionnaire

study to capture the disease profile of food stall patrons may help establish the relationship between crow pathogens and human disease in the area which may help secure Local City Council Authority Funding for crow control.

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#### REFERENCES

- Abe, N., Nagoshib, M., Takamic, K., Sawanod, Y. & Yoshikawae, H. (2002). A survey of *Blastocystis* sp. in livestock, pets and zoo animals in Japan. *Veterinary Parasitology* **106**: 203-212.
- Angus, K.A. (1983). Cryptosporidiosis in Man, Domestic Animals and Birds: A Review. *Journal of the Royal Society of Medicine* **76**(1): 62-70.
- Bangert, R.L., Ward, A.C.S., Stauber, E.H., Cho, B.R. & Widders, P.R. (1998). A survey of the aerobic bacteria in the faeces of captive Raptors. *Avian Diseases* **21**(1): 53-62.
- Cheesebrough, M. (1987). Medical Laboratory Manual for Tropical Countries Volumes I & II. 2<sup>nd</sup> ed. Butterworth-Heinemann, Oxford.
- Chua, K.B., Goh, K.J., Wong, K.T., Kamarulzaman, A., Tan, S.K.P., Ksiazek, T.G., Zaki, S.R., Paul, G., Lam, S.K. & Tan, C.T. (1999). Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. *Lancet* **354**(9186): 1257-1259.
- Dickinson, E.C., Eck, S. & Martens, J. (2004). A preliminary preview of the *Corvidae*. *Systematic Notes on Asian Birds* **350**: 85-109.
- Farizawati, S., Lim, Y.A., Ahmad, R.A., Fatimah, C.T. & Siti-Nor, Y. (2005). Contribution of cattle farms towards river contamination with *Giardia* cysts and *Cryptosporidium* oocysts in Sungai Langat Basin. *Tropical Biomedicine* **22**(2): 89-98.
- Farmer, J.J., Fanning, G.R., Huntley-Carter, G.P., Holmes, B., Hickman, F.W., Richard, C. & Brenner, D.J. (1981). *Kluysvera*, a new (redefined) genus in the family Enterbacteriaceae: Identification of *Kluysvera ascorbata* sp. and *Kluysvera cryocenscens* in clinical specimens. *Journal of Clinical Microbiology* **13**(5): 919-933.
- Fayer, R., Trout, J.M. & Upton, S.J. (2000). Epidemiology of *Cryptosporidium*: Transmission, detection and identification. *International Journal of Parasitology* **30**: 1305-1322.
- Feare, C.J., Sanders, M.F., Blasco, R. & Bishop, J.D. (1999). Canada Goose (*Branta canadensis*) droppings as a potential source of pathogenic bacteria. *Journal of the Royal Society of Promotional Health* **119**(3): 146-155.
- Flemer, K. & Drewes, L. (1988). Species related differences in the incidence of gram-negative bacteria isolated from the cloaca of clinically normal psittacine birds. *Avian Diseases* **32**(1): 79-83.
- Graczyk, T.K., Fayer, R., Trout, J.M., Lewis, E.J., Farley, C.A., Sulaiman, I. & Lal, A.A. (1998). *Giardia* sp. Cysts and Infectious *Cryptosporidium parvum* oocysts in the faeces of migratory Canada Geese (*Branta canadensis*) *Applied Environmental Microbiology* **64**(7): 2736-2738.
- Hopkins, B.A., Skeeles, J.K., Houghten, G.E., Skegle, D. & Gardner, L. A. (1990). Survey of infectious diseases in wild turkeys (*Meleagris gallopavo silvestris*) from Arkansas. *Journal of Wildlife Diseases* **26**(4): 468-472.





- Hussong, D., Damare, J.M., Limpert, R.J., Sladen, J.L., Weiner, R.M. & Colwell, R.R. (1979). Microbial impact of Canada Geese (*Branta Canadensis*) and Whistling Swans (*Cyngus columbianus columbianus*) on aquatic ecosystems. *Applied Environmental Microbiology* **37(1)**: 14-20.
- Ignoffo, C.M. (1992). Environmental factors affecting persistence of Entomopathogens. *Florida Entomology* **75(4)**: 516-525.
- Leelayoova, S., Vithayasai, N., Watana-veeradej, V., Chotpitayasunondh, T., Therapong, V., Naaglor, T. & Mungthin, M. (2001). Intestinal microsporidiosis in HIV-infected children with acute and chronic diarrhea. *Southeast Asian Journal of Tropical Medicine and Public Health* **32**: 33-37.
- Meier, G.M. (2007). *Corvus splendens*. The Global Invasive Species Specialist Group (ISSG) of the IUCN Species Survival Commission. The International Union for Conservation of Nature. Switzerland.
- Ngui, C.Y.K. Hazards of re-zoning. *Malaysian Business* August 16, 2004.
- Rohela, M., Lim, Y.A., Jamaiah, I., Khadijah, P.Y., Laang, S.T., Nazri, M.H. & Nurulhuda, Z. (2005). Occurrence of *Cryptosporidium* oocysts in wrinkled Hornbill and other birds in the Kuala Lumpur National Zoo. *Southeast Asian Journal of Tropical Medicine and Public Health* **36 (4)**: 34-40.
- Sarria, J.C., Vidal, A.M. & Kimborough, R.C (2001). Infections caused by *Kluyvera* species in humans. *Clinical Infectious Diseases* **33**: 63-74.