OCCURRENCE OF BACTERIAL PATHOGENS AND ANTIMICROBIAL RESISTANCE IN PERI-URBAN WILD DOGS

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ABSTRACT

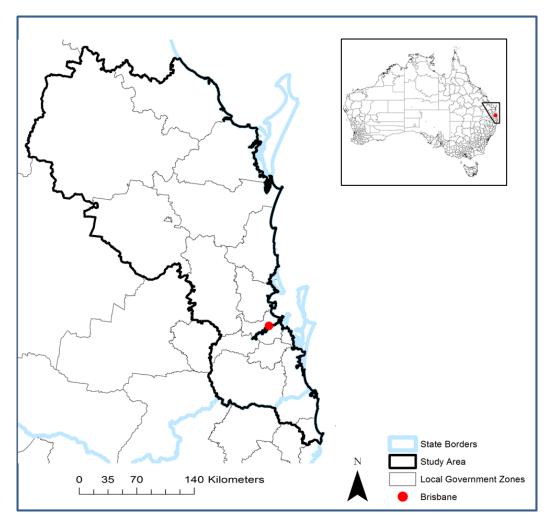
In Australia, wild dogs (Canis familiaris) are common in peri-urban environments, live within a close distance to houses, and visit household backyards and other public high-use areas such as school grounds and parklands. Wild dogs are known to harbour parasites and their presence within these environments has human-health implications. Bacterial pathogens may also affect human health, but the prevalence and diversity of such pathogens in wild dogs remains poorly studied. We examined the presence bacterial pathogens in peri-urban wild dogs to further understand the potential risks to human health. We collected faecal, whole blood, serum and nasal samples from wild dogs captured during council management programs within north-east New South Wales and south-east Queensland. Microbiological, molecular, and serological methods were used to detect and identify targeted pathogens. The results of this study indicate that carriage of bacterial pathogens by peri-urban wild dogs is less prevalent than parasitic pathogens. However, the presence of ampicillin-resistant bacteria remains of public health concern, as wild dogs may spread microbial resistant genes into the environment. The seroprevalence of C. burnetti suggests that wild dogs may be a reservoir of the pathogen and the detection in faeces suggests that they may be a possible source of Q-fever transmission in Queensland. We conclude that bacterial pathogens carried by peri-urban wild dogs pose potential deleterious impacts to public health. However, studies to further confirm these results are required and specific risk analyses are needed within human habitats, including assessing the associations of wild dog diets and other exposure sources.

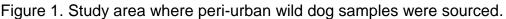
Keywords: Zoonoses, public health, pathogens, wild dog, antimicrobial resistance.

MATERIALS AND METHODS

Study population

Wild dog faecal and blood samples and nasal swabs were collected between August 2012 and May 2015. Wild dogs (n=201) were humanely trapped and euthanised during routine council control programs from regions within south-eastern Queensland and northern New South Wales, within close proximity to human populations (Figure 1).





Isolation of bacterial species

To isolate individual bacterial species, specific methods for each pathogen were applied. See Harriott (2018, pp 44-45) for detailed methods. Tests for each pathogen could not always be conducted on the full sample size (n=201) due to variability in the availability of the appropriate sample type required (e.g. faecal, whole blood, serum, nasal swab).

RESULTS

Ampicillin-resistant *E. coli* was the most commonly isolated bacteria from peri-urban wild dog faeces, detected in 20% of samples tested. One-third of these samples were only resistant to ampicillin, with the remaining resistant to two or more antimicrobials. *Salmonella spp.* was isolated from three samples that were identified as *S.* potsdam. *S.* birkenhead and *S.* subsp 1. Methicillin-sensitive *Staphylococcus aureus* was identified in five samples but methicillin-resistant *S. aureus* was not isolated. In addition, *Brucella suis, Campylobacter spp.* and *Rickettsia felis* were not detected. *Coxiella burnetii* exposure was present in 23.1% of serum samples tested, with a small proportion of samples also revealing shedding in faeces. *Brucella suis, Campylobacter* spp., methicillin-resistant *Stayphylococcus aureus* or *Rickettsia felis* were not detected.

DISCUSSION

Peri-urban wild dogs are being exposed to some zoonotic bacterial pathogens, but their prevalence is not as common as parasitic pathogens (Harriott, 2018). *Escherichia coli* is a naturally occurring and often commensal bacterium found in the gastrointestinal tracts of animals. As a result, we specifically focused on the presence of multi-drug resistant (MDR) *E. coli*. Antimicrobials are essential for the treatment of pathogenic bacteria in both humans and other animals. The presence of genes resistant to antimicrobials in wild populations of animals provides a mechanism to spread these genes in the environment (Allen *et al.*, 2010). The source of peri-urban wild dog exposure to antimicrobial resistant (AMR) bacteria remains unknown. However, wildlife acquire AMR bacteria through contact with anthropogenic sources that pollute the environment with either AMR bacteria or antimicrobials such as farms or human waste (Swift *et al.* 2019). Multiple peri-urban wild dog samples were resistant to two or more drugs and, although the sample size was relatively small, it opens the possibility for future research into this topic.

Infection by Salmonella spp. in peri-urban wild dogs is likely to be opportunistic, as most human cases of Salmonella tend to be sporadic (Stafford and Bell, 2011). The carriage of potentially-pathogenic Salmonella serovars by wild dogs is likely to reflect environmental sources of the pathogen, with infection in wild dogs resulting from predator-prev interactions. However, further research is required to understand the role of wild dogs in maintaining or promoting environmental contamination with Salmonella species. We did not detect the presence of Campylobacter spp. in wild dog faecal samples. However, Campylobacter spp. is difficult to culture and can be sensitive to environmental stressors, so we cannot discount its potential presence in wild dog populations. Other pathogens not detected during the study include Brucella suis and Rickettsia felis. Brucella suis had previously been reported in domestic pig-hunting dogs in New South Wales and Queensland (Johnston, 2016). Therefore, it would be expected that wild dogs exposed to feral pigs could also harbour the pathogen. However, the capacity of dogs (wild and domestic) to act as long-term carriers and as potential sources of infection to humans or other animals is unknown and requires investigation. The DNA of R. felis can degrade quickly in blood samples, with several factors such as extended storage also reducing the ability to extract DNA of high quality. Hence, it is possible that there were limitations in our sampling protocol that inhibited our ability to detect R. felis.

Peri-urban wild dogs are being exposed to *Coxiella burnetii*, the causative agent of Q-fever. Despite having access to a safe and effective vaccine, Australia consistently reports approximately 400-600 new cases of Q-fever annually, with Queensland accounting for about half of those reports (National Notifiable Disease Surveillance System, 2019). Queensland has also had several human infections reported where no direct animal contact had occurred. There is evidence of several wildlife species in Australia having exposure to *C. burnetii* and peri-urban wild dogs in south-eastern Queensland are known to encroach into urban and residential areas. This could be increasing the potential to transmit *C. burnetii* to humans via the excretion of the bacteria into the environment. These results warrant further investigation into the role of wild dogs in the transmission of *C. burnetii*.

The results of this study demonstrate that peri-urban wild dogs can harbor zoonotic bacterial pathogens that pose potential deleterious impacts to public health. Carriage rates of bacterial pathogens by peri-urban wild dogs is less than parasitic pathogens, but nevertheless remain significant. In particular, the presence of ampicillin-resistant bacteria

remains of public health concern, as wild dogs may spread microbial resistant genes into the environment. Future research is required to further examine the level of antimicrobial resistance amongst wild dog populations, and their potential role in the transmission of *C. burnetii* into urban areas. Specific risk analyses assessing the association of wild dog diet and other potential exposure sources would enable greater understanding of transmission routes amongst wildlife populations.

ACKNOWLEDGMENTS

This research was conducted as a postgraduate degree (Harriott, 2018) and was approved by The University of Queensland Animal Ethics committee (Approval number SVS/145/13). These data have also been published in scientific journals (see: Harriott *et al.*, 2019a; Harriott *et al.*, 2019b). Thanks to those who contributed or assisted with sample collection.

REFERENCES

Allen, H.K., Donato, J., Wang, H. H., Cloud-Hansen, K.A. Davies, J. and Handelsman, J. (2010). Call of the wild: antibiotic resistance genes in natural environments. *Nature Reviews Microbiology* 8 251-259 doi:10.1038/nrmicro2312

Harriott, L (2018). Prevalence, risk factors and geographical distribution of zoonotic pathogens carried by peri-urban wild dogs. PhD Thesis, School of Veterinary Science, The University of Queensland, Gatton, Qld

Harriott L., Gentle M., Traub., Soares Magalhães R.J. and Cobbold R. (2019a). The association between diet of periurban wild dogs and zoonotic pathogen carriage. *Australian Mammalogy*, -. doi:10.1071/AM18042

Harriott L., Gentle M., Traub., Soares Magalhães R.J. and Cobbold R. (2019b) Zoonotic and economically significant pathogens of peri-urban wild dogs across north-eastern New South Wales and south-eastern Queensland, Australia. *Wildlife Research*, -. doi:10.1071/WR/18110

Johnston, K. (2016). Brucellosis in dogs guidelines for veterinarians. www.dpi.nsw.gov.au/__data/assets/pdf_file/0008/577376/Brucellosis-in-dogs-guidelines-for-veterinarians.pdf (NSW, Department of Primary Industries).

National Notifiable Disease Surveillance System (2019). Number of notifications of Q-fever, received from State and Territory health authorities in the period of 1991 to 2018 and year to date notifications for 2019. Accessed March 2019. http://www9.health.gov.au/cda/source/rpt_4.cfm

Stafford, R. J. and Bell, R. (2011). Monitoring the incidence and causes of 40 disease potentially transmitted by food in Australia: annual report of the OzFoodNet Network, 2011. Communicable Diseases Intelligence 39, e236–e264.

Swift, B. M. C., Bennett, M., Waller, K., Dodd, C., Murray, A., Gomes, R. L., Humphreys, B., Hobman, J. L., Jones, M. A., Whitlock, S. E., Mitchell, L. J., Lennon, R. J. and Arnold, K. E. (2019). Anthropogenic environmental drivers of antimicrobial resistance in wildlife. *Science of The Total Environment* 649 12-20 doi:10.1016/j.scitotenv.2018.08.180