Zoonotic and economically significant pathogens of peri-urban wild dogs across north-eastern New South Wales and south-eastern Queensland, Australia

Lana Harriott A,B,E, Matthew Gentle B, Rebecca Traub C, Ricardo J. Soares Magalhães A,D and Rowland Cobbold A

ASchool of Veterinary Science, University of Queensland, Gatton, Qld 4343, Australia.
BPest Animal Research Centre, Biosecurity Queensland, Department of Agriculture and Fisheries, 203 Tor Street, Toowoomba, Qld 4350, Australia.
CFaculty of Veterinary and Agricultural Science, University of Melbourne, Parkville, Vic. 3010, Australia.
DChild Health Research Centre, University of Queensland, South Brisbane, Qld 4101, Australia.
E Corresponding author. Email: Lana.Harriott@daf.qld.gov.au

Abstract

Context. Peri-urban wild dogs are known to reside within high-risk and densely populated regions and are capable of harbouring a variety of zoonotic pathogens. Despite recognising the potential of peri-urban wild dogs to carry zoonotic pathogens, limited prevalence data are currently available to assist in understanding the potential risks that peri-urban wild dogs pose within developed communities.

Aims. The aim of the present research was to establish the current status of key zoonotic and economically significant pathogens in peri-urban wild dogs.

Methods. Two hundred and one peri-urban wild dog cadavers were collected from south-eastern Queensland and northern New South Wales. In addition, whole blood, serum and faecal samples were also collected. Pathogens were identified through several morphological, microbiological and molecular methods.

Key results. Helminth parasites were detected within 79.6% of peri-urban wild dogs; Echinococcus granulosus was the most common pathogen, with adult worms being detected within 50.7/6.9% of intestines, followed by Spirometra erinacei (36.6/6.4%); hookworms, including Ancylostoma caninum and Uncinaria stenocephala (28.8/7.1%); Toxocara canis (5.4/3.1%) and Taenia spp., including T. serialis and T. pisiformis (4.5 ± 2.8%). Bacterial pathogens detected included methicillin-resistant Escherichia coli (20.0 ± 10.1%), Salmonella spp. (3.7 ± 4.0%) and methicillin-sensitive Staphylococcus aureus (3.3 ± 2.7%).

Conclusions. The present study is the most comprehensive investigation of zoonotic pathogen carriage in peri-urban wild dogs in Australia. Parasitic infections in peri-urban wild dogs are common, with tapeworms representing the majority of intestinal pathogens. Important zoonotic bacterial pathogens are carried by peri-urban wild dogs, although at a much lower prevalence than are parasites.

Implications. The presence of these pathogens in free-ranging peri-urban dog populations suggests a strong potential for public health risk, most notably from E. granulosus. These data are inherently important as baseline information, which is essential to guide risk-based management of peri-urban wild dog impacts.

Additional keywords: disease, dingoes, parasites, bacteria, Echinococcus granulosus, free-ranging dogs.

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Introduction

Several wildlife species around the world have successfully adapted to peri-urban and urban habitats (Koenig et al. 2001; Prange et al. 2003; Statham and Statham 1997). Peri-urban areas are commonly classified as a transitional zone between urban and rural landscapes (Low Choy et al. 2007). In Australia, wild dogs (Canis familiaris) are free ranging across the majority of the mainland (Fleming et al. 2014), including within urban and peri-urban regions (Allen et al. 2013; McNeill et al. 2016). Peri-urban wild dogs and other urban carnivore species are known to utilise much smaller home-ranges than do their rural counterparts (Bateman and Fleming 2012; McNeill et al. 2016), which suggests that peri-urban areas have a higher resource abundance (Newsome et al. 2013). Importantly, peri-urban wild dogs can remain within 700 m (Allen et al. 2013) or 1000 m (McNeill et al. 2016) of houses or buildings at all
times, as well as frequently utilise and traverse public environments such as recreational ovals, parklands, school yards and residential backyards. This is concerning not only for potential human–wildlife conflicts, including attacks on the public, their pets, livestock animals, and native wildlife, but also introduces the potential for transmission of zoonotic pathogens.

The extent to which peri-urban wild dogs harbour zoonotic pathogens is currently unknown. Previous research has largely focused on domestic household, rural working or pound populations of animals (Palmer et al. 2008; Jenkins et al. 2014a). For many of the common pathogens, the epidemiology, modes of transmission, routes of infection, parasite life cycles and pathogenicity of their infections are well described (Deplazes et al. 2011; Chen et al. 2012). However, the significance of infection in domestic canines is often reduced by the fact that most animals are treated with broad-scale anthelmintic therapy. In Australia, this is also assisted by the requirement and facilitation of owners to remove any faeces left by their dog in public-use areas. As a result, contamination of environmental sources with infectious pathogens is more likely to be attributed to untreated wild populations of animals. Therefore, peri-urban wild dogs could contribute towards the transmission of zoonotic pathogens to humans, either via direct transmission or enhanced epizootic cycling.

Peri-urban regions play a significant role in agricultural production around Australia (Choy and Buxton 2013). Impacts from wild dogs carrying zoonotic pathogens in agricultural settings may result in reduced production levels, morbidity and mortality of livestock and devaluing damaged product at slaughter. Pathogens may be significant to both humans and livestock, but many are specific to each group. Therefore, it remains important to consider both the human-health aspects and potential economic impacts on the livestock industry from peri-urban wild dogs.

The aim of the present study was to investigate the carriage of pathogens by peri-urban wild dogs that are a risk to public health and to livestock industries. Beyond providing valuable estimates of infection burden to assist in evaluating risk, this knowledge is essential in supporting epidemiological studies to inform strategies for management.

Materials and methods

Study population

In all, 201 whole wild-dog carcasses were collected between August 2012 and May 2015 (Harriott 2018). Males and females were equally represented within the dataset at 49% and 51% respectively. Peri-urban wild dogs provided for the present study were trapped either as a part of routine council control programs or private pest-management programs. We classify peri-urban regions as a transitional zone adjacent to, and influenced by, urban centres (Low Choy et al. 2007). They are classified neither as urban nor rural and are comprised of a fragmented mixture of rural, residential and commercial land uses. Trapping locations were influenced by public complaints or active management plans for control of feral animals. Wild dogs were humanely caught with foothold traps. They were captured from south-eastern Queensland (SEQ), a small section of northern New South Wales, and regions just north of SEQ within close proximity to human populations (Fig. 1). Human population density was sourced from the Center for International Earth Science Information Network (CIESIN), Columbia University, 2016; gridded population of the world, version 4 (GPWv4): population density; Palisades, NY: NASA Socioeconomic Data and Applications Center (SEDAC), at http://doi.org/10.7927/H4NP22DQ, accessed 5 November 2018. The present study was completed as part of a postgraduate research program (Harriott 2018) and approved by the University of Queensland Animal Ethics Committee (Approval number SVS/145/13).

Sample collection

Exposure to and identification of zoonotic pathogens was conducted using blood and faecal samples, combined with examination of intestinal tracts. Blood samples were collected via cardiac puncture and transferred to EDTA (EDTA) and serum-separator (SST) vials (Becton, Dickinson and Co., Sydney, NSW, Australia) and kept cool. Blood vials were kept in a portable fridge or on ice (if possible) until fieldwork was completed. EDTA samples were immediately placed in the freezer and SST tubes were refrigerated at 4°C until the serum had separated. Once separated, the supernatant was collected off the SST tubes and stored frozen. Faecal samples were collected from the rectum and stored in three separate tubes, each one containing 10% neutral buffered formalin (NBF), 2.5% potassium dichromate and 20% glycerol. Faeces in formalin and potassium dichromate were stored at room temperature (~23°C) and faeces in glycerol and potassium dichromate were stored at room temperature (~80°C) as soon as possible. After samples had been collected, wild dog cadavers were placed into individual large body bags and frozen, within reasonable time (on the same morning as capture), at ~18°C for later necropsy. At necropsy, the entire small intestine was removed from all dogs (n = 201) and its contents were flushed with water and expelled into a dish. The intestinal contents were then transferred into a disposable container for transport to the laboratory and kept refrigerated (3°C) until examination within 24 h. The intestinal tract was then excised longitudinally and the mucosa was macroscopically examined for the presence of hookworms or other parasites. The upper jaw was removed for the purpose of collecting the two canine teeth to age the wild dogs for future studies. Throughout this process, the nasal cavities were assessed for the presence of tongue worm (Linguatula serrata).

Parasite isolation and identification

Intestinal contents were filtered initially through a 918-μm sieve and washed into a beaker to isolate larger parasites. Parasites were placed into a Petri dish for initial identification, and then stored in 70% ethanol. All adult helminths were identified on the basis of morphological characteristics (Bowman 2013). Contents of the beaker were then washed through a 200-μm-mesh sieve. All items trapped on the mesh were washed into a clean beaker and the total volume was adjusted to 1000 mL. Contents were stirred and two 50-mL subsamples were collected. Each subsample was individually examined in small amounts under a dissecting microscope in a Petri dish marked with ~1-cm squares. Parasites other than those discovered in the coarse-sieving stage were identified. For Echinococcus granulosus,
worms were counted for two 50-mL subsamples as previously described by Jenkins et al. (2008), so as to provide an estimate of the total *E. granulosus* worm burden for each dog. Faecal samples in 10% formalin (n = 156) were washed and were subject to sodium nitrate (specific gravity (SG) 1.20; Inpankaew et al., 2014) and zinc sulfate (SG1.18; Faust et al., 1938) flotation for egg and/or oocyte identification and quantification. The entire coverslip was examined under a light microscope at ×10 and ×40 magnification and species of eggs and/or oocytes were noted.

**Genomic DNA extraction from faecal samples**

Aliquots of ~2 g of faeces (from the potassium dichromate sample) were washed twice with phosphate-buffered saline and centrifuged at 2000g for 3 min at room temperature. A 200-mg subsample of faeces was transferred to an empty 2-mL tube with ~1 g of silica/zirconia 0.5-mm beads (Daintree Scientific, St Helens, Tasmania, Australia) and 370 μL of Mo Bio Powerbead solution from the PowerSoil DNA isolation kit (Mo Bio Laboratories Inc., Carlsbad, California, USA) The remaining DNA-extraction protocol was performed as per the Mo Bio protocol (https://mobio.com/media/wysiwyg/pdfs/protocols/12888.pdf, accessed 1 May 2017) with minor modifications. A volume of 50 μL of C6 solution was used at Step 20 to increase DNA concentration. DNA concentration was measured using a nano-drop 1000 spectrophotometer (Thermo Fisher, Waltham, MA, USA).

**Genomic DNA extraction from blood samples**

DNA was extracted from whole-blood samples collected in EDTA tubes. The DNeasy Blood and tissue kit (Qiagen, Hilden, Germany) was used following the manufacturer’s protocol, with only minor modifications. The blood volume was increased to 200 μL and the final elution volume was decreased to 50 μL for more concentrated DNA. Total DNA concentration was measured and adjusted (if needed) to ~50 ng per 1 μL before polymerase chain reaction (PCR).

**Genomic DNA extraction from worms**

DNA was extracted from adult worms of *Taenia* spp., and morphologically identified according to Bowman (2013). The DNeasy Blood and Tissue kit (Qiagen) was used following the manufacturer’s protocol with minor modifications. Approximately 0.25 g of worm tissue was incubated at 56°C with 180 μL of ATL buffer and 20 μL of proteinase K until completely lysed. The final elution volume was decreased to 50 μL. Total DNA concentration was measured and adjusted (if needed) to ~50 ng per 1 μL before the PCR.
Polymerase chain reaction (PCR)

All DNA samples were screened for eukaryotic DNA to ensure the presence of amplifiable DNA. Samples were tested using universal primers 18SEUDIR 5′-TCTGCCCATGTAASGGTTG-3′ and 18SEUINV 5′-TAATTGCGGCTGCTG-3′ for amplification of a 140-bp fragment of the nuclear 18S gene from eukaryotic DNA (Fajardo et al. 2008). The annealing temperature was modified to 60°C (Wang et al. 2013).

Samples were tested for hookworm by using previously published protocols to amplify a section of the internal transcribed spacer (ITS) regions of *A. caninum*, *A. ceylanicum*, *A. braziliense* and *U. stenocephala* (Traub et al. 2004, 2007). Amplified ITS PCR products were subjected to direct digestions as described in Palmer et al. (2007), so as to differentiate among species. PCR products that were too faint to visualise using restriction fragment-length polymorphism were subjected to purification and bidirectional DNA sequencing for species confirmation by the Animal Genetics Laboratory (School of Veterinary Science, University of Queensland). DNA sequences were analysed on Finch TV 1.4.0 (Geospiza Inc. Seattle, WA, USA) and compared with published sequence data on GenBank using BLAST. Whole-blood samples were tested for spotted fever group rickettsia by amplification of a 297-bp region of the outer membrane protein B (ompB) gene of spotted fever group *Rickettsia* according to Hii et al. (2011). To test for *Neospora caninum*, we used previously published primers and the cycling protocol of Fish et al. (2007).

Isolation of bacterial species

To test for *Salmonella* spp., faecal samples had been stored in 20% glycerol at −80°C until testing. Faeces were swabbed and suspended in 5 mL of Rappaport–Vassiliadis (RV) broth (Oxoid, Altrincham, Cheshire, England) and incubated at 37°C for 18–24 h. A total of 10 µL of the enrichment broth was aspirated and streaked onto brilliant green sulfa agar (BGA) and incubated at 37°C for 18–24 h. Isolates represented by red–pink–white colonies surrounded by a red/pink zone were subcultured onto sheep blood agar (SBA) and MacConkey agar (MCA). All suspect *Salmonella* organisms were subject to a microbact™ test (Oxoid). Identification of serotype was conducted by Queensland Health Forensic and Scientific Services, Brisbane, Queensland. To test for *Campylobacter* spp., faecal samples stored in 20% glycerol at −80°C were transferred using a sterile dry swab, using contact methods that aimed to maximise sample exposure on the basis of the nature of the respective sample, onto modified charcoal cefoperazone deoxycholate agar (Oxoid) and incubated in an atmosphere consisting of 5–6% oxygen, 10% carbon dioxide and 84–85% nitrogen at 41.5°C for 48 h. Detection methods for *Escherichia coli* focussed on the identification of multi-drug resistant strains, as indicators for resistant Gram-negative bacteria. Faecal samples were partially defrosted before using a dry swab and streaking onto ampicillin–MacConkey (AMP–MCA) plates (Oxoid) and incubated at 37°C for 18–24 h. Three suspect colonies were selected and plated individually onto SBA plates, which were again incubated for 18–24 h at 37°C. Growth on SBA plates were subject to rapid™ Spot Indole (Remel, San Diego, CA, USA) and pyrrolidonyl arylamidase (PYR) testing to confirm suspect colonies as *E. coli*. Confirmed *E. coli* colonies were subjected to sensitivity testing. Three to four colonies, per sample, confirmed as *E. coli* were selected and grown in 4 mL of Mueller–Hinton Broth at 37°C for 3–4 h or until denser than 0.5 McFarland standard. Incubated broth was added to 4 mL of saline until equivalent to a 0.5 McFarland standard. Two drops of the solution were dropped onto Mueller–Hinton agar (MH) and streaked evenly across the surface of the plate. Another drop was placed onto SBA for purity testing. Antimicrobial disks were stamped onto the streaked MH plate. Both the MH and SBA plates were incubated at 37°C for 18–24 h. Chosen antimicrobials are commonly used in human or veterinary medicine and included cephalothin (KF), amoxicillin clavulanate 2:1 (AMC), ampicillin (AMP), cefoxitin (FOX), ceftazidime (CAZ), ciprofloxacin (CIP), trimethoprim-sulfamethoxazole (SXT), tetracycline (TE), amikacin (AK), gentamicin (CN), tetracycline (TUL) and florfenicol (FFC) (Oxoid). To test for both meticillin-resistant and -sensitive *Staphylococcus aureus* (MRSA and MSSA), nasal swabs were collected at necropsy and were suspended in 1.5–2.0 mL of Mueller–Hinton broth containing 20% (v/v) glycerol and stored at −80°C. A total of 100 µL of the broth was aspirated and added to 2.0 mL of Mueller–Hinton broth containing 6.5% (w/v) sodium chloride (NaCl) and incubated at 37°C for 18–24 h. To test for MSSA, 10 µL of broth was aspirated and streaked onto mannitol salt agar containing 6.5% (w/v) NaCl. Plates were incubated aerobically at 37°C for 18–24 h. Suspect MSSA colonies were subjected to a Gram stain, catalase test and staph latex-agglutination test for confirmation. To test for MRSA, 10 µL of broth was aspirated and added to 2–5 mL of tryptic soy broth containing 2.5% (w/v) NaCl, 3.5 mg L⁻¹ cefoxitin and 20 mg L⁻¹ aztreonam and incubated at 37°C for 18–24 h. A total of 10 µL of the selective enrichment broth was aspirated and streaked onto brilliance MRSA agar.

Serology

Serum *Brucella suis* testing was performed by Biosecurity Sciences Laboratory, Brisbane (Queensland). Samples underwent an initial rose bengal (RB) test with brucellosis antigen (IDEXX, Westbrook, ME, USA). Positive samples were confirmed using compliment fixation (CF) testing.

Analysis

The prevalence of pathogens and 95% confidence intervals (CI) were calculated for all pathogens. The overall hookworm prevalence was calculated utilising more than one method of detection. The total number of positive samples across any method was divided by the total number of samples tested within all methods (excluding samples that were negative at intestinal sampling and corresponding faecal samples were not available for flotation or PCR to confirm).
Table 1: Number (and percentage) of wild dogs testing positive for parasitic pathogens for each sampling technique

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Samples positive in intestine (n = 201), n (% ± CI)</th>
<th>Samples positive by faecal floatation (n = 156), n (% ± CI)</th>
<th>Samples positive by PCR (n = 82), n (% ± CI)</th>
<th>Samples positive on the basis of combined methods (%) ± CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Echinococcus granulosus</td>
<td>102 (50.7 ± 6.9)A</td>
<td>7 (4.3 ± 3.1)</td>
<td>n.a.</td>
<td>50.7 ± 6.9</td>
</tr>
<tr>
<td>Hookworms</td>
<td>11 (5.5 ± 3.1)</td>
<td>28 (17.2 ± 5.9)</td>
<td>32 (39.0 ± 10.5)A</td>
<td>28.8 ± 7.1</td>
</tr>
<tr>
<td>Ancylostoma caninum</td>
<td>4 (4.9)</td>
<td>n.a.</td>
<td>32 (39.0)</td>
<td>50.7 ± 6.9</td>
</tr>
<tr>
<td>Uncinaria stenocephala</td>
<td></td>
<td>n.a.</td>
<td>4 (4.9)</td>
<td>50.7 ± 6.9</td>
</tr>
<tr>
<td>Spirometra erinacei</td>
<td>73 (36.3 ± 6.4)A</td>
<td>42 (25.7 ± 6.8)</td>
<td>n.a.</td>
<td>36.6 ± 6.4</td>
</tr>
<tr>
<td>Taenia spp.</td>
<td>9 (4.5 ± 2.8)A</td>
<td>7 (4.3 ± 3.1)</td>
<td>n.a.</td>
<td>4.5 ± 2.8</td>
</tr>
<tr>
<td>T. serialis</td>
<td></td>
<td>n.a.</td>
<td>6 (3.0)B</td>
<td>50.7 ± 6.9</td>
</tr>
<tr>
<td>T. pisiformis</td>
<td></td>
<td>n.a.</td>
<td>1 (0.5)B</td>
<td>50.7 ± 6.9</td>
</tr>
<tr>
<td>Taenia spp.</td>
<td></td>
<td>n.a.</td>
<td>2 (1.0)B</td>
<td>50.7 ± 6.9</td>
</tr>
<tr>
<td>Toxocara canis</td>
<td>11 (5.4 ± 3.1)A</td>
<td>7 (4.3)</td>
<td>n.a.</td>
<td>5.4</td>
</tr>
</tbody>
</table>

A PCR conducted on DNA from the adult worm, not faecal sample.

Results

Zoonotic parasites

A total of 79.6% of dogs sampled had a parasitic infection and, of those, 28.1% were infected with multiple species (Table 1). Mixed infections consisted most commonly of *E. granulosus* (n = 40) and *S. erinacei* (n = 29). Two wild dogs were infected with three different parasites, and another two wild dogs were infected with four species of intestinal parasites. *Echinococcus granulosus* was the most common parasite, detected in 50.7% (CI 43.8–57.7) of all dogs (results not shown for *E. granulosus* intensity), followed by *S. erinacei* and *A. caninum* at 36.3% (CI 29.7–43.0) and 28.8% (CI 21.7–36.0), respectively. Adult hookworms were identified within the intestinal tracts of 5.5% of the wild dogs at necropsy. Faecal-floatation methods increased the prevalence to 17.2% and molecular methods further increased prevalence to 39%. All samples that were positive at faecal floatation and had a corresponding sample for PCR were also positive at PCR. Several samples that were negative at faecal floatation returned positive at PCR. Taking into consideration the variations in sample size across the different testing methods, the overall combined hookworm prevalence was 28.8%. Four dogs were also shown to be infected with *Ucinaria stenocephala* and DNA sequences of these isolates showed 99% or greater similarity to previously published sequences of GenBank accession number HQ262054.1 for *U. stenocephala*. *Toxocara canis* and *Taenia* spp. infections were found in 5.4% (CI 2.3–8.6) and 4.5% (CI 1.6–7.3) of wild dogs respectively. Of the nine adult worms of *Ucinaria stenocephala* and DNA sequences of these isolates showed 99% or greater similarity to previously published sequences of GenBank accession number AB731674.1 for *T. serialis* and one of seven to have 94% sequence similarity to previously published sequence of GenBank accession number JN870104.1 for *T. pisiformis*. PCR did not detect *Neospora caninum*. No adult *L. serrata* individuals were detected at postmortem examination.

Zoonotic bacteria

The bacterial pathogens of interest were not commonly identified within the faecal material and nasal passages of peri-urban wild dogs (Table 2). *Salmonella* spp. were found at low levels (3.7 ± 4%), although 20% (CI 9.9–30.1) of faeces sampled (n = 60) contained ampicillin (AMP)-resistant *E. coli*. Of those samples, 33.3% were resistant only to AMP, 41.6% were resistant to AMP plus one other antimicrobial and 25% were resistant to AMP plus two other antimicrobials. The additional antimicrobials to which resistance was demonstrated included AMC, STX, TE and/or KF. *Proteus mirabilis* was also identified in a single faecal sample. *Brucella suis* was not detected within the sampled population. Multiple samples (n = 7) were suspected positive on the RB test, but were all negative at CF. All samples were also negative for *Rickettsia felis* and methicillin-resistant *S. aureus*; however, low levels of methicillin-sensitive *S. aureus* were detected (3.3 ± 2.7%).

Discussion

Given the comprehensive nature of the present study, both in terms of sample size and the number of pathogens screened, some conclusions can be drawn about pathogen infection in wild dogs in a large peri-urban area of eastern Australia. Parasitic infections in peri-urban wild dogs are common. Previous studies have generally focussed on rural populations, where a high prevalence of pathogens such as *S. erinacei* (Coman 1972; Jenkins et al. 2008), *Taenia* spp. (Coman 1972; Jenkins et al. 2014b) and *E. granulosus* (Ballock et al. 1985; Morrison et al. 1988; Jenkins and Morris 1991; Jenkins et al. 2008) has been reported in parasite carriage in wild dogs across Australia. Using a sample of peri-urban wild dogs from south-eastern Queensland (SEQ) and northern New South Wales (NSW), the present study demonstrated that *E. granulosus*, hookworms and *S. erinacei* also commonly infect these populations. Existing studies of *E. granulosus* infection in peri-urban wild-dog populations are discordant in the reported endemicity (Brown and Copeman 2003; Jenkins et al. 2008; Smout et al. 2013). Consistently high temperatures are likely to be major factors...
in the reduced prevalence of *E. granulosus* in northern Australia (Wachira et al. 1991; Torgerson and Heath 2003). Although Jenkins et al. (2008) reported a prevalence somewhat similar to that in the present study, all of the infected dogs were located towards the northern and western regions of Australia, with those closest to residential areas being free of the pathogen. In contrast, our results showed that wild dogs infected with *E. granulosus* are commonly found in the close proximity to residential areas, and *E. granulosus* is broadly distributed throughout SEQ, and parts of north-eastern NSW. The ability of infective eggs to travel and spread via wind, flies and within soil via accidental hosts and fomites (e.g. on shoes) enhances the range of the parasite. This is a significant concern for public health. SEQ is a highly populated area, being home to an estimated 3.4 million people in 2017 (ABS 2018). Within our study area, wild dogs can live within 1000 m of residential houses at all times, and repeatedly traverse roads, suburban backyards and frequently utilised public areas such as school yards and parklands (McNeill et al. 2016). In Switzerland, an emerging epidemic of human alveolar echinococcosis, caused by *Echinococcus multilocularis* (not present in Australia), was linked with a recorded increase in the urban fox population 10–15 years prior (Schweiger et al. 2007). *Echinococcus granulosus* is similar in that in can take several years for the disease to manifest in humans. Although current human cases in Australia are rare, it is important to conduct long-term surveillance to detect any developing impacts from the recent high prevalence detected in peri-urban wild dogs.

The low prevalence of hookworm (5.5%) recorded during postmortem examination increased markedly following further examination using faecal-flotation (17.2%) and molecular (39%) methods. Despite this, the prevalence of hookworms remained much lower than the 70.7% previously detected in scats from far-northern Queensland (FNQ; Smout et al. 2013) and the 74% previously detected in Townsville (Brown and Copeman 2003). Our estimates were also lower than the 37% prevalence in south-eastern Queensland (Jenkins et al. 2008). Climatic conditions such as increased humidity and temperature favour the presence of *Ancylostoma spp*. The reason for the low prevalence of hookworm in our study could be attributed to the ability of *A. caninum*, the most commonly detected species, which undergoes hypobiosis during the colder months, whereas, in tropical regions of Australia, the parasite will shed year round (Gibbs 1982). Hookworms are reportedly common in the domestic-dog population in Australia (Palmer et al. 2008), with the majority of these being *A. caninum* (Palmer et al. 2007). Similarly, *A. caninum* represents the main species detected in wild dogs (Brown and Copeman 2003; Jenkins et al. 2008; Smout et al. 2013). Although detected in wild-dog populations in FNQ (Smout et al. 2013), *A. ceylanicum* was not identified within our study area. Four wild dogs were found to have co-infections of *A. caninum* and *Uncinaria stenocephala*, which has not previously been reported as far north as SEQ. *Uncinaria stenocephala* is generally associated with the southern regions of Australia where temperatures are lower and

| Table 2. The presence of bacterial pathogens in peri-urban wild dogs |

<table>
<thead>
<tr>
<th>Species Test</th>
<th>Tested n</th>
<th>Positive n (% ± CI)</th>
<th>Local government area</th>
<th>Co-infections</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Brucella suis</em></td>
<td>39</td>
<td>0 (0)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Campylobacter spp.</td>
<td>82</td>
<td>0 (0)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ampicillin-resistant <em>Escherichia coli</em></td>
<td>60</td>
<td>12 (20 ± 10.1)</td>
<td>Gold Coast</td>
<td><em>Echinococcus, Toxocara</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Gold Coast</td>
<td><em>Hookworm</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Gold Coast</td>
<td><em>Echinococcus</em></td>
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<td></td>
<td></td>
<td></td>
<td>Gold Coast</td>
<td><em>Echinococcus</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sunshine Coast</td>
<td><em>Hookworm, Spirometra</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Somerset</td>
<td><em>Spirometra</em></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Somerset</td>
<td><em>Echinococcus, Spirometra</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Byron</td>
<td><em>Echinococcus, Hookworm</em></td>
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<td></td>
<td></td>
<td></td>
<td>Byron</td>
<td><em>Echinococcus, MSSA</em></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Byron</td>
<td><em>Echinococcus</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ipswich</td>
<td><em>Echinococcus, Salmonella sp.</em></td>
</tr>
<tr>
<td>MRSA</td>
<td>164</td>
<td>0 (0)</td>
<td>Gold Coast</td>
<td><em>Echinococcus, Hookworm</em></td>
</tr>
<tr>
<td>MSSA</td>
<td>164</td>
<td>5 (3.3 ± 2.7)</td>
<td>Gold Coast</td>
<td><em>Echinococcus, Spirometra</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Gold Coast</td>
<td><em>Echinococcus</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Gold Coast</td>
<td><em>Echinococcus</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sunshine Coast</td>
<td><em>Hookworm, Spirometra</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Byron</td>
<td><em>Echinococcus, E. coli</em></td>
</tr>
<tr>
<td><em>Rickettsia felis</em></td>
<td>82</td>
<td>0 (0)</td>
<td>Ipswich</td>
<td><em>Echinococcus, hookworm, E. coli</em></td>
</tr>
<tr>
<td><em>Salmonella spp.</em></td>
<td>82</td>
<td>3 (3.7 ± 4.0)</td>
<td>Byron</td>
<td>Hookworm</td>
</tr>
<tr>
<td>S. Birkenhead</td>
<td></td>
<td></td>
<td>Gold Coast</td>
<td>Unknown tapeworm</td>
</tr>
</tbody>
</table>
winters, in particular, are colder (Palmer et al. 2007). Dogs from our study with *U. stenocephala* were all trapped either in the month of May or August, which had respective average temperature ranges (minimum–maximum) of 13.9–24.0°C and 9.8–23.3°C (Brisbane: http://www.bom.gov.au/climate/data/index.shtml?bookmark=200, accessed May 2017), well within the ideal temperature for larval development of *U. stenocephala* (Gibbs and Gibbs 1959). Educating adults on the transmission routes of hookworm (percutaneous infection) and appropriate hygiene (no bare feet in parklands and hand-washing after children playing) as well as the clinical signs in humans (pruritic self-resolving rash, abdominal signs) is likely to contribute significantly to our ability to mitigate the potential risk of hookworm infections posed by wild dogs.

Two species of *Taenia* (*T. serialis, T. pisiformis*) were detected in the present study. Urbanisation may limit the exposure of wild dogs to pathogens that are more frequent in livestock than wildlife. The intermediate stages of *T. serialis* and *T. pisiformis* are commonly found in rabbits and are probably more likely to be detected among the peri-urban wild-dog population than are those of *T. hydatigena* and *T. ovis*, which use cattle, sheep and goats as intermediate hosts (Arundel 1972). Humans are accidental intermediate hosts for both species of *Taenia* detected in the present study, although records of cases are very rare (Ing et al. 1998). Therefore, the presence of *Taenia* species within the peri-urban wild-dog population is unlikely to be of concern to public health or be of major economic significance for livestock industries.

The high prevalence of *S. erinacei* that we have reported in the peri-urban wild-dog population could reflect its prevalence in feral-pig populations, which could impact on the international trade of feral-pig meat through condemnations of infected carcasses. Most feral-pig meat is exported to Europe, with exports between 2006 and 2007 being responsible for approximately AU$13 million into the economy (Bengsen et al. 2014). Feral-pig meat is commonly consumed in aboriginal communities around Australia (Koichi et al. 2012), but consumption by the general Australian public is generally very low and the local commercial market is small. Human infection can also result from consumption of pleurocercoids in the muscles of other intermediate or paratenic hosts (e.g. tadpoles, frogs, toads) or through accidental consumption of procercoids within copepods in fresh water (Lee et al. 1990). However, people in peri-urban regions have access to treated drinking water and often carry bottled water when spending time outdoors. As a result of this, as well as Australia’s rigorous meat-hygiene inspection and food-safety assurance systems, public health implications from *S. erinacei* are likely to be minimal. However, profitability for the sector may be affected.

*Toxocara canis* was found at low numbers within the intestines of the peri-urban wild dogs. These results are similar to previous findings on the Sunshine Coast (Jenkins et al. 2008). However, prevalence was lower than that that detected in foxes in Western Australia, where 14.9% were infected (Dybing et al. 2013). Geographic and demographic risk factors of both human (Congdon and Lloyd 2011) and canine (Regis et al. 2011) toxocarosis are well described; however, spatial factors and environmental distribution of infective eggs are not as well understood. Although young dogs in Bristol were found to shed the highest number of eggs, their movements were limited and, hence, contribution to environmental contamination was primarily through fox faeces (Morgan et al. 2013). This suggests that animals that shed the highest number of eggs may not be the most significant factors in the spread and/or maintenance of pathogen if their spatial movements are limited. Within the current study area, foxes (*n* = 102) were found to have a prevalence of *T. canis* (8.8%) similar to that in wild dogs (L. Harriott, unpubl. data). The low prevalence in both the fox and wild-dog populations may be from a lack of infective eggs in the environment (owing to an increase in anthelmintic use in domestic dogs) or a lack of paratenic hosts in the diet. There is also the potential for age-related immunity to be present because most infections in dogs greater than 6 months old could be somatic (Claerebout et al. 2009). Despite this, the potential impacts on human health are substantial and should be recognised by pest managers and health authorities.

The variety of parasitic species co-occurring in sampled dogs was lower than that in similar studies, and unlike previous reports, there were no uncommon parasites detected (Jenkins et al. 2008; Dybing et al. 2013). Peri-urban wild dogs across SEQ are mostly exposed to similar dietary items and exhibit similar preferences (Allen et al. 2016), and food items are usually readily available. Abundant and available food items may reduce the need for dogs to explore outside of their home range, and consume additional food resources that are outside of their preferred prey species. Hence, the exposure to different environmental sources of pathogen is probably limited.

Our results indicated that carriage of zoonotic bacterial pathogens is uncommon. As *E. coli* is a common bacterium than can be found naturally in the gastrointestinal tract of all animals and is frequently commensal rather than pathogenic, our analyses were focussed on detecting dogs that carried multi-drug-resistant (MDR) *E. coli*. In each of the local government areas north, south and west of Brisbane, at least one dog was found to be carrying MDR *E. coli*, although the Brisbane City local government area did not show any positive samples. Multiple samples were resistant to two or more drugs and, although the sample size was small, the presence of bacteria resistant to one or several antimicrobials in the peri-urban wild-dog population proves a significant risk to human health, because it enables the spread of resistance genes and bacteria into the environment and to other species of animals. It suggests value in future research into this topic. We also recorded three peri-urban wild dogs infected with *Salmonella*. Stafford and Bell (2011) suggested that many human *Salmonella* cases tend to be sporadic infections rather than outbreaks, which our preliminary data would also support for peri-urban wild-dog populations. *Staphylococcus aureus* has been detected in domestic dogs, which are often infected with lineages of human origin, rather than the dog-specific clonal complex (Malik et al. 2006; Strommenger et al. 2006; Weese et al. 2006). Although the number of detected wild dogs carrying MSSA was small, it warrants further investigation into MSSA and MRSA carriage by peri-urban wild dogs, because the potential public-health implications are significant.

There are some limitations to the present study. First, sampling could not be considered random. The collection of
wild dogs was influenced by public complaints or existing wild-
dog management programs. Second, the logistics of field
collection and storage of samples (particularly blood) made it
difficult to refrigerate and process samples quickly. This may
have reduced our ability to detect some pathogens, particularly
bacteria. There were also discrepancies between the testing
methods, suggesting that parasites, at least, are often best
identified by locating the adult within the definitive host.
However, molecular and faecal-floatation methods were more
sensitive in the detection of hookworms than was identifying
adults within the intestines. Hookworms are small and fragile,
and the freeze–thaw process may have affected the ability to
detect adults. Finally, temporal aspects of epidemiology are not
accounted for in here, although sampling was conducted
throughout the year. These limitations are minor because they
are unlikely to affect the interpretation of the data or the overall
conclusions.

Our results provided estimates of pathogen prevalence in peri-
urban wild-dog populations in eastern Australia, which will
support the strategic management of wild-dog impacts in
these areas (Fleming et al. 2014). Public awareness of health
in domestic dogs (e.g. correct deworming protocols), and
communication on the importance of hygiene (particularly for
trappers), can assist in mitigating the risk posed by pathogens
carried by the peri-urban wild-dog population. Future research
should focus on the risk posed by individual pathogens and their
management, including multi-drug-resistant strains. This could
include environmental screening for these same pathogens
within public areas utilised by peri-urban wild dogs.

Conflicts of interest
The authors declare no conflicts of interest.

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