Carriage of critically important antimicrobial resistant bacteria and zoonotic parasites amongst camp dogs in remote Western Australian indigenous communities

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Camp dogs in indigenous communities in the Western Australian Kimberley Region, share the domestic environment with humans and have the potential to act as carriers of, and sentinels for, a wide range of zoonotic agents, including intestinal parasites and antimicrobial resistant bacteria. In this study, we investigated the carriage of extended-spectrum-cephalosporin-resistant (ESC-resistant) Escherichia coli, methicillin-resistant Staphylococcus aureus (MRSA) and species of hookworm and Giardia among camp dogs in remote Western Australian Aboriginal communities. A total of 141 canine faecal samples and 156 nasal swabs were collected from dogs in four communities of the Western Australian Kimberley region. Overall, ESC-resistant E. coli was detected in 16.7% of faecal samples and MRSA was isolated from 2.6% of nasal swabs. Of most significance was the presence of the community-associated Panton-Valentine leucocidin (PVL)-positive MRSA ST93 and ST5 clones and ESC-resistant E. coli ST38 and ST131. The most prevalent zoonotic intestinal parasite infection was Ancylostoma caninum (66%). The prevalence of Giardia was 12.1%, with the main genotypes of Giardia detected being dog specific assemblages C and D, which are unlikely to cause disease in humans.

Greater than 60% of emerging human infectious diseases are zoonotic1. Companion animals, such as dogs, have been shown to be sources of zoonoses2, in part due to close, prolonged contact with humans and integration into environments of increased human population density. Parasitic and bacterial zoonoses are commonly found in household pets3. Recently, the prevalence of multidrug resistant Escherichia coli resistant to extended spectrum cephalosporins (ESCs), methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-resistant Staphylococcus pseudintermedius (MRSP) in domestic pets has been increasing4–9. Gram-negative bacteria resistant to critically important antimicrobials (CIAs) such as carbapenems and ESCs are of significant public health concern due to limited therapeutic options and the ability of such antimicrobial resistance to be transferred to sensitive Gram-negative bacteria via horizontal gene transfer10,11. It is possible that companion animals may serve as reservoirs for ESC-resistant E. coli due to the close associations between humans and pets12,13. Studies have reported human and companion animal isolates sharing the same genes and displaying identical clonal lineages14–17, suggesting transmission of the bacteria between household pets and humans.

MRSA is a zoonotic and zooanthroponotic agent identified among companion animals and a global health issue. The first human community-acquired MRSA (CA-MRSA) infections were reported in Australian Aboriginal and native Canadian communities in the 1990s18,19, and studies have also reported evidence of MRSA transmission between human and companion animals20–22.

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In addition to the growing threat from CIA resistant bacteria, the public health threat from zoonotic parasites requires close monitoring, particularly among isolated communities of low socioeconomic standing and with poor health education. *Ancylostoma caninum* is a zoonotic canine hookworm species and is one of the soil-transmitted helminths. The most common route of hookworm infection is via penetration of the skin by hookworm larva. As such, communities where the parasite is endemic, coupled with behavior of walking bare-foot, increases the risk of infection. Zoonotic infection with *Ancylostoma caninum* can result in cutaneous larva migrans, a creeping, itchy rash caused by migrating larvae under the skin.

*Giardia* is a cause of gastrointestinal infection which is common in humans around the world, particularly in developing regions, and a high prevalence of *Giardia* infection among indigenous communities in Western Australia has been documented. Clinical manifestations include diarrhea, nausea, and abdominal pain and distension, and serious sequelae in infants and children include failure-to-thrive syndrome.

Previous studies into zoonotic intestinal parasites from dogs in remote Aboriginal communities in the Kimberley region of Western Australia were conducted over 20 years ago, and data on antimicrobial resistant (AMR) bacteria in companion animals in these communities is lacking. This study provides a contemporary examination of the zoonotic parasites and antimicrobial resistant bacteria from camp-dogs in Aboriginal communities in the Kimberley region.

**Results**

**Carriage of ESC-resistant *E. coli*.** ESC-resistant *E. coli* was isolated from 12.8% (18/141) of faecal samples, with the majority coming from the West Kimberley communities (12 of 21 isolates). The 18 positive faecal samples yielded, in total, 21 ESC-resistant *E. coli* isolates.

The isolates were defined by multi-locus sequence-type (MLST) with most isolates belonging to sequence type (ST) 38 (42.8%, n = 9). The remaining isolates were characterised as ST2144 (9.5%, n = 2), ST131 (9.5%, n = 2), ST1408 (4.3%, n = 1), ST3520 (4.3%, n = 1), ST1569 (4.3%, n = 1), ST68 (4.3%, n = 1), ST3268 (4.8%, n = 1), ST106 (4.8%, n = 1) and ST872 (4.8%, n = 1). The two ST131 isolates were identified in the East Kimberley 2 (EK2) region while the ST2144 isolates were from West Kimberley 1 (WK1) region. As a result of low sequence coverage, the STs for two isolates could not be determined.

All ESC-resistant *E. coli* isolates were resistant to two or more classes of antimicrobials (multidrug resistant) with universal resistance to ceftriaxone and ampicillin. Two of the isolates from EK2, (ST38 and ST131), and one ST3268 isolate from East Kimberley 3 (EK3) demonstrated resistance or intermediate resistance to ciprofloxacin. The ST38 and ST131 isolates were also resistant to trimethoprim/sulfamethoxazole. Two ST38 isolates, from different locations, were resistant to six or more antimicrobial classes and carried corresponding resistance genes.

The two phenotypically and genotypically distinguishable ST38 STs were identified in the Kimberley region. Based on molecular detection results of the species of interest, 82 dogs were infected with hookworm and *Giardia* combined. A total of 111 dogs were found to be infected with one or more parasites based on combined molecular and microscopic examination. Overall, hookworm infection was the most common with 93% of 141 dogs (66%) positive. The other helminths observed in the samples were *Toxocara canis* (4.3%, n = 6), *Spirometra erinaceid* (3.5%, n = 5), *Taenia spp.* (1.4%, n = 2), and *Spirocercus lupi* (0.7%, n = 1).

*Giardia* was the second most prevalent protozoa in dogs (12.1%, n = 12) after *Isospora spp.* (12.7%, n = 18) followed by *Sarcocystis spp.* (9.9%, n = 14). The prevalence of *Giardia* and hookworm in each community is shown in Table 3. EK1 had the highest crude prevalence of hookworm (93.8%) and *Giardia* (18.8%).

Most dogs from which sequencing was successful (12/17 dogs, 70.6%) were carrying *Giardia Assemblage C* (GenBank accession numbers: MP974555-MP974558; MP974560; MP990014- MP990016; MP769400). Three Isolates; B1 HC10 (GenBank accession number: MP990017), K2–27 (100% similar to GenBank accession number: KY797492) and B1 HC34 (GenBank accession number: MP990018) were identified as assemblage D at the *gdh* locus. Evidence suggesting mixed populations was found in two isolates, B1 HC23 and B1 HC7. Isolate B1 HC23 was identified as assemblage D at the *gdh* locus and assemblage C (GenBank accession number: MP974560) at the pti locus. The sequence of Isolate B1 HC7 at the *gdh* locus matched representative GenBank accessions for...
Genotypic and phenotypic antimicrobial resistance profile of ESC-resistant \textit{E. coli} isolates collected from dog faeces in remote Australian communities. \textbf{EK} (East Kimberley), \textbf{WK} (West Kimberley), \textbf{SXT} (Trimethoprim/Sulfamethoxazole), \textbf{TET} (Tetracycline), \textbf{FOX} (Cefoxitin), \textbf{CRO} (Ceftriaxone), \textbf{CN} (Gentamicin), \textbf{AMP} (Amoxicillin), \textbf{SXT} (Sulfamethoxazole/Trimethoprim), \textbf{CIP} (Ciprofloxacin), \textbf{TMP} (Trimethoprim).

Discussion

The current study provides information on the carriage of ESC-resistant \textit{E. coli}, MRSA and zoonotic enteric parasites among camp dogs that are in close contact with Western Australian Aboriginal communities. ESC-resistant \textit{E. coli} were carried by 16.7\% of dogs with some of the isolates belonging to the globally disseminated ST131 and ST38 ESC-resistant pandemic clones. Additionally, camp dogs were colonized by PVL-positive CA-MRSA (ST93 and ST5) clones at a low prevalence (2.6\%); and zoonotic intestinal parasites \textit{Giardia} and \textit{Ancylostoma caninum} were present at prevalences of 12.1\% and 66\% respectively. It should be noted that the prevalences reported are crude, given the opportunistic nature of sampling, and that the numbers of dogs present in each region are only estimates, with no valid enumeration data available.

ST38 was identified as the major \textit{E. coli} ST in the faecal samples collected in two community locations. ST2144 and ST131 were also identified for more than one isolate. The two ST2144 were isolated from the same animal in the West Kimberly community. This sample grew 3 morphologically distinguishable colony types, however whole genome sequencing showed that only two separate genotypes were present. ST2144 carrying \textit{bla}_{CTX-M} genes, which was isolated from two animals located in the East Kimberley community, can exist as a globally disseminated multi-drug resistant pandemic extra intestinal pathogenic \textit{E. coli} responsible for causing variety of extra intestinal infections in humans, including urinary tract infection and bacteremia\textsuperscript{31,32}. Significantly, ST131 and ST38 have previously been reported as causes of disease in various animals, including dogs\textsuperscript{33–36}. The limitations of this study do not allow conclusions to be drawn on how the sampled animals acquired these infections. It could be hypothesized that they naturally circulate in dogs in these communities or alternatively they are spillover from the human population. However, these findings are of public health concern, given the possibility that these clonal types may be transferred from dogs to humans, and a larger scale study inclusive of human sampling may aid in determining the ecology of resistant \textit{E. coli} in these populations.

PVL-positive ST93 -IV and PVL-negative ST5 -IV are community acquired (CA)-MRSA that have also been found in animals\textsuperscript{37,38}. ST93-IV is the dominant CA-MRSA clone across Australia in humans, and has been associated with a range of skin and soft tissue infections, as well as severe invasive infections such as necrotizing pneumonia\textsuperscript{39–41}. The three MRSA isolates harbored the beta-lactamase gene (\textit{blaZ}), the penicillin-binding protein, PBP 2a gene (\textit{meCA}) and the efflux pump gene (\textit{norA}). This finding is of important public health significance in these populations, as these isolates are resistant to beta-lactam antibiotics which may be used for treatment of pneumonia.
skin and ear infections that are highly prevalent among Aboriginal communities. As for *E. coli*, a more detailed study to examine the ecology of these MRSA clones in Aboriginal communities and camp dogs is warranted.

The prevalence of *Ancylostoma caninum* in dogs identified in the current study is similar to a previous report from the same area, completed in 1993. The high prevalence of *A. caninum* increases the opportunity for spread of the infection to humans in the communities, which can cause cutaneous larva migrans. Although *A. caninum* is a zoonotic agent, it is considered of minor public health significance as this species of hookworm rarely progresses past cutaneous infections. Dog Health Programs in Aboriginal communities that were first introduced in the Kimberley region of Western Australia in 1992, used the anthelmintic Ivermectin to reduce the prevalence of scabies and hookworm in dogs. Unfortunately, the treatment was only able to reduce the intensity of the infection but did not significantly diminish the prevalence of canine hookworm. The failure of eradication of the parasite might be correlated to periodic treatment, however, more recently dogs in these communities have been treated with more frequent dosing.

### Table 2. Molecular characteristics and phenotypic antimicrobial resistance profiles of nasal MRSA isolates collected from dogs in remote Australian communities. EK 1 (East Kimberley, Community 1), EK 2 (East Kimberley, Community 2), WK 1 (West Kimberley, Community 1), FOX (cefoxitin), P (penicillin).

| Isolate | Origin | Sequence type | Resistance genes | Phenotypic antimicrobial resistance | Virulence Factors |
|---------|--------|---------------|------------------|------------------------------------|-------------------|
| B1 B19  | EK 1   | ST5           | blaZ, mecA, norA | FOX, P                             | *aur, splB, splA, sen, sak, lukF-PV, lukS-PV, hlb, hlgB, hlgA, hlgC, lukD, lukE, eddmA, seg, sen, seu, sei, sem, seo* |
| B1 B21  | EK 1   | ST93          | blaZ, mecA, norA | FOX, P                             | *splA, aur, splE, scn, sak, lukS-PV, lukE, hlb, hlgA, hlgC, hlgB, hlgD, lukF-PV, lukD* |
| B2 B61  | WK 1   | ST93          | blaZ, mecA, norA | FOX, P                             | *splA, splE, aur, sen, sak, hlb, lukF-PV, lukS-PV, lukE, lukD, hlgA, hlgC* |
| B1 HC17 | EK 2   | ST872         | blaZ, mecA, norA | FOX, P                             | *splB, au, splA, sen, sak, scn, lukD, lukE, hlb, hlgB, seq, sek, hlgA, hlgC* |

### Table 3. Prevalence of *Giardia* and hookworm in canine faeces collected in the Kimberley region.

| Origin | Ancylostoma caninum Prevalence (95% CI) | Giardia spp. Prevalence (95% CI) |
|--------|-----------------------------------------|----------------------------------|
| EK 1   | 15/16 93.8% (71.7, 98.9) 3/16 18.8% (6.6, 43.0) |
| EK 2   | 46/71 64.8% (53.2, 74.9) 9/71 8.5% (6.8, 22.4) |
| EK 3   | 12/26 46.2% (28.8, 64.5) 2/26 7.7% (2.1, 24.1) |
| WK 1   | 20/26 76.9% (57.9, 90.0) 3/26 11.5% (4.0, 29.0) |
| WK 2   | 0/2 0% (0.00, 65.8) 0/2 0% (0.00, 65.8) |
| Total  | 93/141 66% (57.8, 73.3) 17/141 12% (7.7, 18.5) |

### Table 4. *Giardia* assemblages identified in dog faeces based on combined sequencing data from *gdh* and *tpi* genes. NS: No sequence determined.

| Isolate | Gdh Assemblage | tpi Assemblage | Combined result |
|---------|----------------|----------------|-----------------|
| B1 B12  | C              | C              | C               |
| B2 B12  | C              | C              | C               |
| B2 B18  | C              | NS             | C               |
| B2 B25  | NS             | C              | C               |
| B1 B5   | C              | C              | C               |
| B1 B7   | C              | C              | C               |
| B1 HC10 | D              | NS             | D               |
| B1 HC23 | D              | C              | D+C             |
| B1 HC34 | D              | NS             | D               |
| B1 HC7  | C+D            | A              | C+D+A           |
| K1–2   | C              | NS             | C               |
| K1–5   | C              | NS             | C               |
| K1–31  | C              | C              | C               |
| K1–34  | C              | C              | C               |
| K1–41  | C              | C              | C               |
| K2–13  | C              | C              | C               |
| K2–27  | D              | NS             | D               |

*skin and ear infections that are highly prevalent among Aboriginal communities.*

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The prevalence of *Ancylostoma caninum* in dogs identified in the current study is similar to a previous report from the same area, completed in 1993. The high prevalence of *A. caninum* increases the opportunity for spread of the infection to humans in the communities, which can cause cutaneous larva migrans. Although *A. caninum* is a zoonotic agent, it is considered of minor public health significance as this species of hookworm rarely progresses past cutaneous infections. Dog Health Programs in Aboriginal communities that were first introduced in the Kimberley region of Western Australia in 1992, used the anthelmintic Ivermectin to reduce the prevalence of scabies and hookworm in dogs. Unfortunately, the treatment was only able to reduce the intensity of the infection but did not significantly diminish the prevalence of canine hookworm. The failure of eradication of the parasite might be correlated to periodic treatment, however, more recently dogs in these communities have been treated with more frequent dosing.
received 3-monthly moxidectin treatments. As such these results are of concern, and may indicate anthelmintic resistance or heavy environmental contamination by dogs which have missed regular treatments.

The prevalence of *Giardia* infection in dogs in this study (12.1%, 95% CI 7.7, 18.5) was similar to findings in the same region over 20 years ago (17%)\(^27\) and to a national study of gastrointestinal parasites of dogs in Australia (9.3%, 95% CI 7.8–10.8)\(^45\). This study found that the genotype of *Giardia* from dogs in the region were mostly canine-specific Assemblages C and D. The zoonotic *Giardia* Assemblage A was only found in one sample, and it would appear that the likelihood of transmission of *Giardia* between dogs and humans in the Kimberley Region remains low.

The management of dogs is of paramount importance in minimizing the spread of zoonotic agents through these communities. Dogs in this study were able to roam freely, and scavenged on human waste. Access to materials such as human faeces has the potential for dogs to become infected with human associated bacterial clones and parasites, and maintain them in the community. Ongoing de-sexing and treatment clinics together with continuous client education regarding good husbandry practices and correct anthelmintic, antiprotozoal and antibiotic administration are also important to prevent recurrent infections.

In conclusion, this study demonstrates the carriage of antimicrobial resistant bacteria and zoonotic enteric parasites amongst camp dogs in remote Western Australian communities. The carriage of human associated MRSA (ST93 and ST5) and ESC-resistant *E. coli* (ST131 and 38) identified in this study is of particular importance, and requires further study to determine whether there is movement of CIA resistant bacteria from humans to animals and the potential for zoonotic transmission to humans.

### Methods

#### Study area.

The Kimberley Region is in the north of Western Australia. The region is a remote area populated by Aboriginal communities. The samples were collected from three communities in the East Kimberley and two communities in the West Kimberley. Available information about total dog population and number of samples in each community are presented in Table 5.

#### Source of isolates.

Work undertaken in this survey was approved by the Murdoch University Animal Ethics Committee (Permit #408 and #R2876/16), with all experiments performed in accordance with relevant guidelines and regulations. Nasal swabs were collected from 156 dogs from five communities. Of these dogs, faecal samples could be collected from 141, with the remaining having empty rectums. Sampling was conducted for diagnostic purposes by the Murdoch University veterinary team undertaking a neutering operation and dog health program in the Kimberley region in three-time periods: June 2016, October 2016 and June 2017. Sample numbers were based solely on dogs entering the neutering programme. Only dogs which had not been previously neutered were sampled to prevent resampling the same individual. Faecal samples were collected into standard 70 ml plastic containers. Nasal swab samples were collected using swabs into charcoal media (Copan, Italy). Samples were stored at 4 °C until processed.

#### Bacterial Isolation and detection.

For MRSA isolation, swabs were plated onto Brilliance MRSA Agar (ThermoFisher Scientific) and incubated overnight at 37°C. Colonies resembling MRSA were subcultured onto 5% Sheep Blood Agar (Edwards Media). Screening for ESC-resistance was performed by incubating the faecal samples onto Brilliance ESBL Agar (ThermoFisher Scientific) and incubating overnight at 37°C. Colonies resembling ESBL *E. coli* were sub–cultured onto 5% Sheep Blood Agar (Edwards Media). If more than one colony morphology was identified on a plate an isolate from each colony type was taken. Identification of all isolates was conducted using a Bruker microflex MALDI-TOF.

#### Antimicrobial susceptibility testing.

Isolates underwent susceptibility testing via disc diffusion according to the Clinical Laboratory Standards Institute (CLSI) Performance Standards for antimicrobial disk susceptibility tests M02-A12\(^46\). MRSA were tested using the following seven antimicrobials: trimethoprim/sulfamethoxazole, tetracycline, cefoxitin, erythromycin, penicillin, ciprofloxacin and gentamicin. *E. coli* isolates were tested using the following 12 antimicrobials: Trimethoprim/Sulfamethoxazole, tetracycline, cefoxitin, ceftriaxone, gentamicin, chloramphenicol, ampicillin, streptomycin, imipenem, ciprofloxacin, amoxicillin–clavulanate and meropenem. Zone diameter results were categorized as susceptible, intermediate and resistant using the clinical interpretative criteria specified in CLSI performance standard VET01-S3\(^47\). If interpretive criteria was not present in VET01-S3, CLSI performance standard M100-S25 was used\(^48\).

#### Detection of resistance genes.

DNA was extracted from isolates using a MagMax DNA multi sample kit (ThermoFisher Scientific) as per manufacturer's instructions with the modification to omit the RNase treatment step. Library preparation was performed using an Illumina NexTera XT library preparation kit as per

| Origin             | Approximate population size of dogs | Dogs sampled |
|--------------------|-------------------------------------|--------------|
| East Kimberley (EK) 1 | 30                                  | 16           |
| East Kimberley (EK) 2 | unknown                             | 71           |
| East Kimberley (EK) 3 | unknown                             | 26           |
| West Kimberley (WK) 1 | 100                                 | 26           |
| West Kimberley (WK) 2 | 60                                  | 2            |

Table 5. Canine faecal samples by community and population.
Giardia ples found positive for on qPCR. An approximately 733 bp portion of the glutamate dehydrogenase (gdh) locus was amplified using a protocol previously described by Yang et al.51. Conventional PCR amplification of the gdh locus used the primers GDH1F and GDH2R described by Sulaiman et al.52,53. For this nested PCR, primers GDH2F and GDH3R were used in the primary reaction, and primers GDH4F and GDH4R were used in the secondary reaction (Table 2). PCR reaction volume for each sample in both primary and secondary PCRs was 20 µL consisting of 12.5 µL GoTaq® Green Master Mix (Promega,USA), 0.25 µM of each primer, 6.25 µL nuclease-free water and 5 µL of template genomic DNA. The thermocycling conditions consisted of a pre-heating step at 94 °C for 2 min, followed by 40 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min, and a final extension of 72 °C for 7 min. PCR products were viewed under a 1.5% agarose gel dyed with SYBR®Safe DNA gel stain.

The presence of Giardia in all samples were screened at the glutamate dehydrogenase (gdh) locus using a quantitative PCR (qPCR) procedure previously described by Yang et al.51. Conventional PCR amplification of the glutamate dehydrogenase (gdh) and the triose phosphate isomerase (tpi) locus was conducted on all samples found positive for Giardia on qPCR. An approximately 733 bp portion of the gdh gene was obtained using formerly published primers52,53. For this nested PCR, primers GDH1F and GDH2R were used in the primary reaction, and primers GDH4F and GDH4R were used in the secondary reaction (Table 2). PCR reaction volume for each sample in both primary and secondary PCRs was 20 µL containing 10 µL GoTaq® Green Master Mix (Promega,USA), 0.25 µM of each primer, 4 µL nuclease-free water and 5 µL of template genomic DNA. Cycling conditions for primary PCR were 1 cycle of 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s with a final extension of 72 °C for 7 min and a 12 °C hold. The cycling conditions for the secondary PCR were similar to the primary PCR, except the annealing temperature, which was 52 °C. PCR of the tpi locus utilised a nested PCR protocol developed by Sulaiman et al. (2003) with slight modifications54. Primary and secondary primers are shown in Table 6. The predicted PCR product sizes of primary and secondary reactions were 605 bp and 530 bp, respectively. The PCR reaction for the primary reaction comprised of: 10 µL GoTaq® Green Master Mix (Promega,USA), 0.25 µM of each primer, 4 µL nuclease-free water and 5 µL of template DNA. The secondary reaction contained: 12.5 µL GoTaq® Green Master Mix (Promega,USA), 0.25 µM of each primer, 6.25 µL nuclease-free water and 5 µL of DNA. The following cycling conditions were used for both primary and secondary PCRs: 1 cycle of 94 °C for 2 min, followed by 35 cycles of 94 °C for 45 s, 60 °C for 45 s and 72 °C for 1 min.

| Target          | Primer name   | Sequence                                      | Reference |
|-----------------|---------------|-----------------------------------------------|-----------|
| Ancylostoma spp. | RTH1F         | 5′-GATGAGCATTGCGTGAATGCGG-3′                  | 50        |
|                 | RTHW1R        | 5′-GCAAGTGGTTCGAGCAAAG-3′                     |           |
| Giardia gdh     | Primary reaction | GDHF 5′TCAACGTYAAYCGGYTTCCGT-3′              | 52,53     |
|                 | Secondary reaction | GDH2 5′-ACCTCGTCTGRTGGGCGCA-3′              |           |
|                 |                | GDH4 5′-GTTGGCGCARGGCTGATGCA-3′              |           |
|                 | qPCR           | gdhF1 5′-GGGCAAGTCGACACAGA-3′                | 51        |
|                 |                | gdhR1 5′-GCACATCTCCTCCAGGAAAGTACG-3′         |           |
|                 |                | Probe 5′-TCATGGCTCTTGCCAG BHQ2-3′           |           |
| Giardia tpi     | Primary reaction | AL3545 5′-AAATTATGGCTGCTCGTG-3′             | 54        |
|                 | Secondary reaction | AL35465′-CAAACCTTTTCGCCAAAACC-3′           |           |
|                 |                | AL3544 5′-CCCTTATCGGGGTAACCT-3′             |           |
|                 |                | AL3545 5′-GTGGCCACCCACCCGTCGCC-3′           |           |

Table 6. PCR primers for Ancylostoma spp. and Giardia.
with a final extension of 72 °C for 7 min. The amplified DNA products from the gdh and tpi PCR were visualized on a 1.5% agarose gel containing SYBR®Safe DNA gel stain.

PCR products of the *Ancylostoma spp*, *Giardia gdh* and *Giardia tpi* reactions were excised from gels and purified using the Wizard®SV Gel and PCR Clean-Up System (Promega, USA) before DNA sequencing. DNA sequencing was performed at the Australian Genome Research Facility (Perth, WA).

Following screening by qPCR for *Giardia*, only samples which were positive upon Sanger sequencing on the *gdh* and/or *tpi* assays were considered as confirmed positives. *Ancylostoma* positive status was also based on Sanger sequence positive PCR results.

**Data availability.** The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

**Accession codes.** B1 B12 gdh (accession number: MF 990015), B1 B12 tpi (accession number: MF 974557), B2 B18 gdh (accession number: MF 990016), B2 B25 tpi (accession number: MF 974558), B1 B5 gdh (accession number: MF 769400), B1 B5 tpi (accession number: MF 974555), B1 B7 gdh (accession number: MF 990014), B1 B7 tpi (accession number: MF 974556), B1 HC10 gdh (accession number: MF 990017), B1 HC23 tpi (accession number: MF 974560), B1 HC34 gdh (accession number: MF 990018), B1 HC7 tpi (accession number: MF 974559).

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Author Contributions
B.R. performed microscopic and molecular analysis on parasites of all samples, interpreted data and wrote manuscript. S.A. and M.A.O. designed the study, were responsible for project oversight and assisted in manuscript preparation. R.J.A. carried out bacterial isolation, assisted in parasite screening and helped to evaluate and edit the manuscript. S.M. performed bacterial isolation. T.L. conducted bacterial Isolation, antimicrobial susceptibility testing and detection of resistance genes. I.D.R. collected samples, assisted in project development and manuscript preparation. A.A. assisted in parasite identification and manuscript preparation. G.W.C. assisted in manuscript preparation and provided analysis of MRSA data.

Additional Information
Competing Interests: The authors declare no competing interests.

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