Endo-parasites of public-health importance recovered from rodents in the Durban metropolitan area, South Africa

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Background: Parasite infections of public health importance carried by Rattus spp. on the African continent (excluding toxoplasmosis) have not been adequately researched. The aim of this study was to investigate endoparasites of public health importance, particularly those associated with R. norvegicus, at different locations and seasons within the port-city, Durban.

Methods: Four hundred rodents (379 R. norvegicus, 10 R. rattus and 11 Mastomys natalensis) were live-trapped at 60 sites in four locations, during wet and dry seasons in 2009. Rats were humanely euthanased, cardiac blood drawn (for blood smears and serology), ectoparasites removed and dissected. Each organ was separately processed to collect parasites. Binary logistic regression and four-way ANOVAs were used to test for the effects of location, season, rodent age and gender on parasite prevalence, richness and abundance.

Results: Eight parasites of public health importance were detected: Gongylonema sp. (25.3%), Trypanosoma lewisi (22.8%), Hymenolepis diminuta (17.2%), Angiostrongylus cantonensis (15.3%), Toxoplasma gondii (11.2%), Moniliformis moniliformis (9.5%), Calodium hepaticum (2.6%) and H. nana (0.8%). Ascaris spp. (probably A. lumbricoides) ova, assumed to have been acquired from consuming infected human faeces were found in rat faeces (4.8%). Parasite species richness was positively associated with location, season and rodent age. Location, season, rat age and gender differentially affected prevalence and worm abundance of parasite species.

Conclusions: These occurrence data of parasites of public health importance provide valuable information to local and provincial organisations and medical practitioners for diagnoses of possible zoonoses, and a reference point for further studies in metropolitan areas of Africa.

Keywords: helminths, parasites, protozoa, public health, Rattus norvegicus, rodents, zoonoses
abundance and richness. Finally, we assessed risk factors for rat to human disease transmission in the urban landscape.

**Methods**

**Study locations and seasons**

To capture rats across the eThekwini metropolitan area, the study area was divided into four locality types, namely: central business district (CBD), harbour (HBR), informal settlements/slums (IS) and urban/peri-urban (U/PU) (Figure 1). There were 60 collection sites across these four locations. The CBD and HBR were characterised by many closely juxtaposed buildings, heavy human traffic and widespread food trade, whereas IS was characterised by slums, informal settlements and low-cost housing developments, and U/PU by formal settlements (i.e. houses and apartments), de-centralised shopping areas, recreational and small-wildlife parks, small poultry farms, and wastewater treatment works. The study spanned one year to allow for seasonal variations. There were two distinct periods of rainfall: five wet months (January, February, October, November and December) and seven dry months (March to September). Climate data were provided by weather-station number 461, Mount Edgecombe, 29°42’0” S, 31°2’0” E, 96 m above sea level.

**Sampling of rodents**

Ethical approval for this study was granted by the Animal Ethics Committee of the University of KwaZulu-Natal (Ref. 031/09/Animal) with the proviso that euthanasia was performed by trained mammalogists in accordance with international ethical guidelines. For logistical and safety reasons, rodents were trapped by eThekwini Health Department’s Vector Control Division. Trapping was largely opportunistic and often done in response to complaints from the public. Custom-made traps resembling the Monarch Rat Trap were used, with bread, vegetables and meat as bait.

Chloroform was used to euthanase each animal before a cardiac puncture was performed to obtain blood. Thin and thick blood smears were made and serum was harvested and frozen for serological tests (plague and leptospirosis by the National Health Laboratory Services (NHLS), Johannesburg, South Africa). Any remaining sera were returned to our laboratory by NHLS to test for toxoplasmosis antibodies. Thin blood smears were fixed in 100% methanol and thick smears were air-dried for one hour. The former were stained with May-Grünwald/Giemsa, allowed to dry and then stored in wooden slide boxes until examination.

Rodents were weighed with a Pasola scale (to the nearest 0.5 g), gender and breeding status recorded, and selected body measurements taken (to the nearest 0.1 mm): total body length (body + tail length), tail length, length of right ear and right hind foot (excluding and including claw). Rodents were then dissected: the diaphragm was removed first, followed by the heart and lungs, the liver, the GIT, the kidneys and bladder and lastly the tongue. Organs (except GIT) were placed into separate jars, covered with digestive medium for tissue (5 g pepsin + 7 ml hydrochloric acid, in 1 l distilled water) and incubated at 37 °C for 12 to 18 h to free parasites for collection, preservation and identification. The GIT was divided into five sections: oesophagus, stomach, small intestine, caecum and large intestine. Each of these sections was carefully slit open, placed in a jar and covered
with 70% ethanol to preserve all parasites in that section. All macroscopically visible acanthocephalans, cestodes and nematodes were removed from the small intestine and preserved in 70% ethanol. Smaller worms of these three helminth taxa and those buried in the mucosa of oesophagus and stomach were removed from each section using a dissecting microscope, identified, counted and placed in 70% ethanol in separate containers for each host animal.

Faeces excrated during euthanasia or found in the rectum at dissection, were placed in 10% formal saline. For rats that had no faecal pellets, a caecal sample was taken and processed as for the faecal pellets. Samples were processed using the modified formal-ether concentration method\(^\text{18}\) to check for helminth eggs and protozoan cysts/oocysts. Adult worms from the caecal sub-samples were stored for future work on parasites of no public health importance.

### Aging of rodents

Because the prepatent periods of most parasites are four to six weeks, prevalence of parasites in very young rats (unweaned pups) are likely to differ from weaned juveniles. Thus, *R. norvegicus* were categorised into three age-groups: pups, juveniles and adults. *Rattus rattus* by-catches were classified into age groups according to Hirata and Nass.\(^\text{19}\) Pups included unweaned rats up to approximately four weeks; juveniles included rats of approximately five to 10 weeks, probably weaned and some sexually mature; and adults were all sexually mature and > 10 weeks old. *Mastomys natalensis* are difficult to age yet those ≤ 30 g typically are juveniles.\(^\text{20}\) In this study, all *M. natalensis* by-catches were adults (>30 g), hence those > 50 g were categorised as old adults and those < 50 g as young adults.

### Identification of parasites of zoonotic importance

**Detection of Toxoplasma gondii**

*T. gondii* infection was determined serologically using the Bio-Rad Pastorex\(^\text{24}\) Toxo kit (USA). All weak positives and inconclusive results on initial testing were re-run and confirmed using a second observer. Results were reported as either positive or negative.

**Zoonotic protozoans and helminths**

*Trypanosoma lewisi,* was identified morphologically according to Hoare.\(^\text{21}\) To identify the acanthocephalan, the taxonomic key by Van Cleave, which uses the armament of the procorbic, was followed.\(^\text{22}\) Cestodes were identified based on the morphology of their scolices and eggs. Nematodes in the liver were identified by broken pieces of gravid female worms and characteristic eggs. Nematodes in oesophagus and stomach mucosa were identified to genus by characteristic scutes at anterior end of the body.\(^\text{23}\) Adult nematodes in the heart and its associated vessels and eggs, and L1 larvae in the lungs, were identified using morphological features as described by Macherras and Sandars.\(^\text{24}\)

**Genetic confirmation of *A. cantonensis***

DNA was isolated from tissue samples using a DNeasy\(^\text{26}\) DNA isolation kit (QIAGEN Inc., USA). Analyses were based on the mitochondrial cytochrome oxidase 1 (CO1) gene (primers: LCO (forward) 5'-GGTCAACAAATCTAAGATATTG and HCO (reverse) 5'-TAACTTCAAGGTGACACAGATATCA) and nuclear ribosomal DNA ITS2 region (primers: NC1 (forward) 5'-ACGCTGTTCCAGGGTTTGT and NC2 (reverse) 5'-TATATGTTTATTTCCCTCCGCT). PCR amplifications were performed in 25 μl volumes. Each reaction contained 0.8 μl sterile water, 2.5 μl 10 X reaction buffer (SuperTherm, UK), 4 μl 25 mM MgCl\(_2\) (SuperTherm, UK), 0.5 μl 10 mM deoxynucleoside-triphosphate mixture (dNTPs) (Roche Diagnostics, Switzerland), 0.2 μl Taq polymerase (5 U/μl) (SuperTherm, UK) and 4 μl of each primer (6 μM) (forward and reverse) per reaction. The thermal cycling parameters used were as follows: CO1 — 94 °C for 4 min, followed by 40 cycles of (95 °C for 1 min, 50 °C for 1 min and 72 °C for 2 min) and followed by 72 °C for 10 min; ITS2 — 95 °C for 5 min, followed by 40 cycles of (95 °C for 1 min, 58 °C for 1 min and 72 °C for 90 s) and followed by 72 °C for 10 min.

Target fragments were purified from excised gel bands using the QIAquick\(^\text{27}\) Gel Extraction Kit (QIAGEN Inc., USA) and sequenced at InqabaBiotec, South Africa. All fragments were sequenced in both directions to allow reconciliation of ambiguous positions. They were aligned using the CLUSTAL W option\(^\text{28}\) of the BioEdit program (ver. 5.0.9 for Windows 95/98/NT) and by visual inspection. Similar sequences were identified by BLAST searches of the NCBI GenBank and downloaded for inclusion in the analyses. For the ITS analysis, the following GenBank sequences were included: *Angiostrongylus cantonensis* EU636007, GQ181112, HQ540543, HQ540544, HQ540547; *Angiostrongylus daskalovi* KX242346; *Angiostrongylus vasorum* EU627592, EU627593 - EU627596; outgroups *Aeluropstrus abstrusus* DQ372965, JX948745. Sequences used in the CO1 analysis were: *Angiostrongylus cantonensis* GQ398121, KT947978; *Angiostrongylus malayensis* KT947979; *Angiostrongylus vasorum* GQ982872, JX268542; *Angiostrongylus costaricensis* KR827449, GQ398122; outgroups *Caenorhabditis briggsae* EU407785 and *Dictyocaulus viviparus* JX519460.

Trees were constructed using the neighbour-joining and parsimony methods in PAUP 4.0b10.\(^\text{29}\) The software jModelTest 0.1.1\(^\text{30}\) was used, applying the AKAIE information criterion, to determine the most appropriate evolutionary model (GTR+G) to use in neighbour-joining analyses. For parsimony analysis, the addition sequence was random, with one tree held at each step and with ten replicates. A total of 1000 bootstrap replicates were carried out for both parsimony and neighbour-joining analyses.

**Examination of faecal pellets for parasite ova**

Helminth eggs and larvae of parasites normally parasitising rats were reported as absent or present on a plus-scale of 1–4. Only eggs from parasites not normally infecting rodents (i.e. those mechanically transmitted via ingestion and excretion) were counted.

### Statistical analyses

Two-way ANOVAs were used to test differences in numbers of *R. norvegicus* captured between location and season. Binary logistic regression was used to identify the most significant predictors - location, season, age and gender - of parasite infection (prevalence). Four-way ANOVAs on the ranked data were used to examine differences in (i) mean worm abundance of zoonotic helminths identified, (ii) mean intensity of trypanosome infections and (iii) species richness, in *R. norvegicus* among location, season, age and gender. All statistical analyses were performed using SPSS (version 23, College Station, Texas, USA).

### Results

**Rodents trapped per location and season**

*Rattus norvegicus* comprised 94.8% (*n* = 379) of the 400 rodents sampled. Additionally, 10 *R. rattus* and 11 *M. natalensis* were trapped. Numbers of *R. norvegicus* trapped per location were: 101 from CBD, 93 from HBR, 97 from U/PU and 88 from IS. There was a significant difference in number of *R. norvegicus* sampled...
among locations and between seasons (2-way ANOVA: $F_{(7, 378)} = 22.136; p < 0.001$). Post-hoc Tukey tests showed that significantly more *R. norvegicus* were trapped at CBD than IS ($p < 0.001$) and U/PU ($p = 0.001$). Further, significantly fewer rats were captured during the wet months ($n = 137$) than dry months ($n = 242$) ($p < 0.001$). Abundance of *R. norvegicus* was highest in September ($n = 75$) and lowest in December ($n = 2$). *Rattus rattus* and *M. natalensis* were captured only at U/PU ($n = 8$ and 11, respectively; in both seasons) and IS [2 *R. rattus* (in both seasons) and 1 *M. natalensis* (in dry season only)].

**Parasites of public health importance**

Patent infections of *Trypanosoma lewisi* (Protista), *M. moniliformis* (Acanthocephala), *H. diminuta* and *H. nana* (Cestoda); *Gongylonema* sp., *A. cantonensis* and *C. hepaticum* (Nematoda), were found in *R. norvegicus* (Figures 2 and 3), while *T. gondii* (Protista) was confirmed serologically. *Rattus rattus* harboured only *T. gondii*, *H. diminuta* and *A. cantonensis*, and *M. natalensis* was infected with *H. diminuta*, *C. hepaticum* (Figure 2) and *Angiostrongylus cantonensis*.

**Figure 2:** Adult worms and tissue pathology (scale bars = 100 μm) 1: Proboscis of *M. moniliformis* showing characteristic rows of recurved hooks; 2: *H. nana* scolex with 4 suckers and raised rostellum armed with hooklets; 3: *H. diminuta* scolex showing 4 cup-shaped suckers and unarmed rostellum; 4: Rear end of *A. cantonensis* male worm showing copulatory bursa and long spicules; 5: *A. cantonensis* female worm with the characteristic “barber’s pole” appearance; 6: *Gongylonema* head end showing characteristic scutes; 7: Liver of male *M. natalensis* at dissection showing the extent of his *C. hepaticum* infection (yellow lesions in the surface parenchyma).

**Figure 3:** Helminth eggs and larva & the protozoan blood parasite found (scale bars = 10 μm). A: *M. moniliformis* egg; B: *H. nana* egg; C: *H. diminuta* egg; D: *A. cantonensis* egg; E: *A. cantonensis* L1 larva; F: *Gongylonema* sp. egg; G: *C. hepaticum* egg; H: *Trypanosoma lewisi* trypomastigotes; I: *Ascaris* sp. egg.

**Figure 4:** (A) Neighbour-joining tree based on 367 nucleotides of the nuclear ribosomal ITS2 DNA region illustrating relationships between experimental samples CK61, CK170 and SAS74, *Angiostrongylus* sequences downloaded from the GenBank, and the outgroups *Aleurostrongylus abstrusus*. Bootstrap support for nodes is given as [nj%/p%] for neighbour-joining (nj) and congruent parsimony (p) analyses and (B) Neighbour-joining tree based on 611 nucleotides of the mitochondrial cytochrome oxidase 1 gene illustrating relationships between experimental samples CK170 and SAS74, *Angiostrongylus* sequences downloaded from the GenBank, and the outgroups *Dictyocaulus viviparus* and *Caenorhabditis briggsae*. Bootstrap support for nodes is given as [nj%/p%] for neighbour-joining (nj) and congruent parsimony (p) analyses.
The CO1 and ITS2 alignments were trimmed to 611 and 367 nucleotides, respectively, and used in further analyses. Analyses based on both the CO1 and ITS2 regions (Figures 4(A) and (B)) were congruent and indicated that the experimental samples were previously unreported haplotypes of *Angiostrongylus cantonensis*. This is based on the inclusion of the experimental samples in a very strongly supported clade (99–100% bootstrap support) which included GenBank samples of *A. cantonensis*. This enabled the experimental samples to be referred to *A. cantonensis* according to the phylogenetic species concept.28

No adult worms were found in some *R. norvegicus* although eggs of *M. moniliformis, H. diminuta* and Gongylonema sp. and L1 larvae of *A. cantonensis* were detected in the faeces. Other eggs found were: *Calodium hepaticum* (ingested via environmental contamination or through necrophagy), *Taenia* sp., *Schistosoma mansoni* and Ascaris sp. (for which rats are not natural hosts). *Toxocara* sp. ova were found in the faeces of one *R. rattus* individual and *C. hepaticum* ova were found in the faeces of the same *M. natalensis* that harboured a patent *C. hepaticum* infection. This was considered as mechanical transmission (meaning: ‘the transmitter is not infected in that tissues are not invaded and the agent does not multiply’).29 Ova of *Moniliformis moniliformis, H. diminuta*, and Gongylonema sp., and L1 larvae of *A. cantonensis* were mechanically transported by seven, 51, 11 and seven *R. norvegicus*, respectively.

Most of the helminth eggs of public health importance that did not infect rodents, but were mechanically transported by them, were the roundworm *Ascaris* sp., probably *A. lumbricoides* (Figure 3). These eggs were present in the gut contents of 20 rats: three rats in U/PU (range of 1–6 eggs/rat); four rats in CBD (1 egg each); and 13 in IS (range of 2–287 eggs/rat).

**Influence of location, season, rodent age and gender on parasite prevalence, richness and abundance**

Because of low sample sizes, endoparasite infections in *Rattus rattus* and *M. natalensis* were not statistically compared with those in *R. norvegicus*.

Sero-prevalence of *T. gondii*

Prevalence of *T. gondii* among *R. norvegicus* was 11.3% (*n* = 14/124) and among *R. rattus* it was 12.5% (*n* = 1/8). In terms of age groups, 16% (*n* = 4/25) were pups, 6.1% (*n* = 3/49) were juveniles and 14.0% (*n* = 7/50) were adults. No *M. natalensis* (*n* = 5) tested positive. Logistic regression showed that location, season, rat age and gender, were not significant predictors for *T. gondii* prevalence (all *p* > 0.05).

**Patent helminth infections**

*Gongylonema* sp. was found in 25.3% of *R. norvegicus* (*n* = 96/379), but was absent from the other two rodent species. This nematode was more prevalent in HBR and CBD than in IS and U/PU (Table 1, Figure 5). Prevalence increased with the age of rats (Table 1). Mean worm abundance was highest in HBR and CBD and lowest in IS, and it increased with the age of rats, particularly in the wet season (Table 2).

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**Figure 5**: Prevalence (Mean ± 95% CI) of *T. lewisi, M. moniliformis, H. diminuta, A. cantonensis, Gongylonema sp.*, and *C. hepaticum* in *R. norvegicus* across 4 locations (CBD = central business district; HBR = harbour; IS = informal settlements; U/PU = urban/peri-urban areas) in Durban.
Prevalence of *Trypanosoma lewisi* was 22.8% (n = 86/378), and was highest in CBD and juveniles (Table 1). Mean intensity of infection was affected by location, age and gender (Table 2).

Prevalence of *H. diminuta* in *R. norvegicus* was 17.2% (n = 65/379; Figure 5), in *R. rattus*, 30% (n = 3/10) and in *M. natalensis*, 36.4% (n = 4/11). *R. rattus* positives were from U/PU. One juvenile (dry season) was infected with six *H. diminuta* and two adults (wet season) with two *H. diminuta* each. Positive *M. natalensis* were adults and from U/PU, three that were caught in the dry season harboured three, eight and two *H. diminuta*, respectively, and one caught in the wet season harboured six worms. Location, season and rat age were significant predictors of *H. diminuta* prevalence and abundance in *R. norvegicus* (Tables 1 and 2).

Prevalence was significantly higher at CBD and HBR than at IS and U/PU (Figure 5); in the wet than in the dry season; and in adults and juveniles than in pups. Highest abundances were found in juveniles at HBR and U/PU and in adults at HBR. Juveniles had high mean worm burdens in both seasons, yet adults had high mean worm burdens principally in the wet season. The few infected pups had very low worm burdens.

Prevalence of *A. cantonensis* was 15.0% (n = 57/379) in *R. norvegicus* and 10% (n = 1/10) in *R. rattus*. The two *M. natalensis* (18.2%; n = 2/11) infections were possibly *A. sandarsae* given that this species was previously recorded from *Mastomys natalensis* in Kenya and Mozambique.

Location, season and rat age were significant predictors for *A. cantonensis* prevalence in *R. norvegicus* (Table 1) – prevalence was significantly higher at IS and U/PU than HBR (Figure 5), in the wet season than dry season, in adults than juveniles and pups, and in juveniles than pups. Mean worm abundance of *A. cantonensis* was significantly influenced by location, season and age as well as interactions between location and season, location and age, and season, age and gender (Table 2). Highest worm abundance was at IS and U/PU.

### Table 1: Significant statistical results for binary logistic regression testing the influence of age and gender of *Rattus norvegicus*, location and season on prevalence of endoparasites

<table>
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<tr>
<th>Parasite</th>
<th>$\chi^2$</th>
<th>df</th>
<th>$p$-value</th>
<th>Variable</th>
<th>Significant variables</th>
<th>$p$-value</th>
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<td>Age</td>
<td>J &gt; A by 2.85x</td>
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<td><em>A. cantonensis</em></td>
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<td>A &gt; P by 105.85 x</td>
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Notes: A = adults; J = juveniles; P = pups; CBD = central business district; HBR = harbour; IS = informal settlements; U/PU = urban/peri-urban areas.

‘Example of how to read ‘Significant variables’ column: ‘CBD > IS by 6.4x’ means rats in CBD are 6.4x more likely to be infected than rats in IS.

Mean worm abundance of *A. cantonensis* was significantly influenced by location, season and age as well as interactions between location and season, location and age, and season, age and gender (Table 2). Highest worm abundance was at IS and U/PU.
Table 2: Significant statistical results for 4-way ANOVAs testing differences in trypanosome intensity of infection, worm abundance and species richness between locations and seasons and among age groups and gender of R. norvegicus

<table>
<thead>
<tr>
<th>Parasite/Spp. Richness</th>
<th>F (df)</th>
<th>p-value</th>
<th>Significant variables</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. lewisi</td>
<td>F (3, 364) = 2.166</td>
<td>&lt;0.001</td>
<td>Location</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Age</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Location*Age</td>
<td>0.022</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Location*Gender</td>
<td>0.046</td>
</tr>
<tr>
<td>M. moniliformis</td>
<td>F (3, 364) = 1.411</td>
<td>0.048</td>
<td>Location</td>
<td>0.046</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Age</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Location*Age</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Season*Age</td>
<td>0.021</td>
</tr>
<tr>
<td>H. diminuta</td>
<td>F (3, 364) = 3.848</td>
<td>&lt;0.001</td>
<td>Location</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Season*Age</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Age</td>
<td>0.018</td>
</tr>
<tr>
<td>Gongylonema sp.</td>
<td>F (3, 364) = 2.085</td>
<td>&lt;0.001</td>
<td>Location</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Age</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Location*Season</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Season*Age</td>
<td>0.005</td>
</tr>
<tr>
<td>A. cantonensis</td>
<td>F (3, 364) = 4.939</td>
<td>&lt;0.001</td>
<td>Location</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Season*Age</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Season*Gender</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Location*Gender</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Species richness</td>
<td>F (3, 364) = 2.895</td>
<td>&lt;0.001</td>
<td>Location</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Age</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

PU in the wet season (alone and combined). Juveniles had the highest worm abundance in PU, and juveniles and adults had the highest worm abundance in the wet season. Male adult rats in the wet season had the highest mean worm abundance.

Prevalence of M. moniliformis in R. norvegicus was 9.5% (n = 36/379; Figure 5). Neither R. rattus nor M. natalensis were infected. The only significant predictor for M. moniliformis prevalence was age; prevalence was significantly higher in adults than in juveniles and pups (Table 1). Mean worm abundance was significantly influenced by the interaction of location and season (Table 2) where the highest mean worm abundance was during the wet season at HBR and IS.

Erichthemobius nana was recovered from three R. norvegicus only at HBR in the wet season. One pup had 49 worms (prevalence 0.9%), one juvenile had eight worms (co-infected with H. diminuta; prevalence 0.7%), and one adult had a single worm (prevalence 0.8%). No H. nana infections were found in the other two rodent species.

Species richness
The model for species richness was significant for location, season and age (as independent effects). Multiple infections were significantly low at IS and in the dry season and increased with age of rats (Table 2).

Discussion
This study is the first detailed account of endoparasites of public health importance from urban rats, principally Rattus norvegicus, in Africa. Similar to previous studies in eThekwini, R. norvegicus was the most common rodent sampled (94.8% of the total catch) and it harboured eight zoonoses. Globally, this rodent species often occurs with other Rattus species and is not always the dominant species, especially in Asia. The eight parasite species of public health importance are cosmopolitan. Location, season and age were significant predictors of parasite species richness – significantly low at informal settlements and in the dry season and increasing with age of hosts. By contrast, neither site nor host age influenced parasite richness in R. norvegicus in Kuala Lumpur, Malaysia, yet parasite species richness in R. rattus, was higher at wet markets and in older rats.

Prevalence of Gongylonema sp. in rodents was relatively high (25.3%). A study in Kuala Lumpur found only 0.4% of R. rattus was infected (no R. norvegicus); one study in India found 17.5% Rattus spp. infected; and, one study in San Juan found 35% Rattus spp. infected. Specimens will be identified to species level based on the gongylonemids in future work. Among 47 recognised species of Gongylonema, 15 are found in rodents, including G. neoplasticum and G. pulchrum. By 2013 there were 57 reported cases worldwide of human infections with Gongylonema spp. (and mostly assigned G. pulchrum), yet the first genetically confirmed case of G. pulchrum was in 2013. Infection is acquired through ingestion of infected intermediate hosts such as beetles or cockroaches, which in human cases, usually occurs unintentionally. Human gongylonemiasis creates the sensation of something moving in the buccal mucosa and some patients removed worms from their mouths. Infected patients complained of reflux (which indicates possible involvement of the oesophagus) and high fever with digestive disturbances and vomiting. Future work should combine genetic identification with morphology to identify the source of human infections and clarify the role that rats play in the transmission of this parasite.
Trypanosoma lewisi had the second highest prevalence (22.8%) among parasites reported in this study. Prevalence and intensity of infection were influenced by location, age, and gender with significantly more rats infected and with highest parasite burdens in the central business district in juveniles and in females. By contrast, prevalences ranged from 1.5% in Malaysia\(^7\) to 21.7% in Brazil.\(^8\) In the latter study, prevalence was highest in the wet season, in males and in young rats. *Xenopsylla cheopis*, the plague flea, is the common vector in warm climates\(^9\) (also the most prevalent on the rats in this study\(^1\)), and infection is by ingestion of flea faeces or fleas.\(^{40}\) Two African studies, one in Nigeria and another in selected sites in three African countries (Tanzania, Swaziland and Namibia), found 75.7%\(^{41}\) and 45.2%\(^{42}\) *R. rattus* respectively, infected with *T. lewisi*. This haemoflagellate can prove fatal in humans\(^{43}\) and in young rats.\(^{44}\) Human cases (often involving infants), have been reported from India, Thailand, Malaysia and Gambia.\(^{45}\) Common symptoms are fever, coughing, anorexia, depression and lassitude.\(^{45}\)

*Hymenolepis diminuta*, a common cosmopolitan parasite of rats and mice, was the only parasite found in all three rodent species. This tapeworm has been found across the globe at prevalences of up to 66.7% in *Rattus* spp.\(^{45}\) Often, human cases in developing countries are incidentally reported when mass faecal parasite surveys in school children are carried out. Clinical case reports by 2004 were < 500, with most cases in Southeast Asia probably due to the cultural practice of entomophagy.\(^{46}\) We found that location, season and age group were significant predictors of both *H. diminuta* prevalence and worm abundance. Although *H. diminuta* has a relatively short prepatent period of 18–20 days, *R. norvegicus* pups were the least affected (prevalence = 3.8%). Potential intermediate hosts for *H. diminuta* include fleas (*Xenopsylla cheopis*), and flour beetles (*Tenebrio molitor*) that are often found in grain storage facilities.\(^{5}\) The latter may explain the high prevalence of *H. diminuta* at the harbour and central business district. Significantly high infections after weaning in the wet season may occur because rats become independent and more likely to encounter abundant intermediate hosts while exploring and foraging.

Prevalence of *Angiostrongylus cantonensis* in *R. norvegicus* is the first from Africa.\(^{47}\) Rats are the definitive host, *A. cantonensis* does not occur naturally in other rodents. This nematode was first discovered in *R. norvegicus* and *R. rattus* in Canton (now Guangzhou), China in 1933,\(^{48}\) and has since been found globally. Location, season and age had significant effects on prevalence and worm abundance of *A. cantonensis*, where highest prevalence and abundance were in adults at informal settlements and urban/peri-urban areas during the wet season. These factors were probably mediated by the intermediate hosts – terrestrial snails and slugs – of *A. cantonensis*. In support, species of the snail families Achatinidae and Helicidae and slugs of the families Uroculidae and Veronicellidae are more commonly found in sub-urban and rural areas than built-up city and harbour areas, especially in the wet season.\(^{49}\) Achatinids are eaten by humans in West Africa,\(^{50}\) yet information on snail-eating in southern Africa is lacking. Slime trails containing infective L1 larvae, left by molluscs on vegetables, are also a source of infection.\(^{51}\) By 2010, there were more than 2 877 human case reports worldwide, the most common symptom being eosinophilic meningitis, but pathology varied from mild to severe, sometimes resulting in death.\(^{51}\) It is frequently encountered in humans in China and the Far East due to their penchant for strange and exotic culinary delicacies.\(^{52}\)

Seroprevalence of the most ubiquitous parasite, *T. gondii*, was 11.3%. One reason for the lack of statistical differences in the number of *T. gondii*-positive rats among location, season, or age groups could be because infections become established within seven days in the rodent host. Although cats, the definitive hosts of *T. gondii*, are common in informal settlements, the status of toxoplasmosis in these cats is not known, nor how inhabitants interact with them. Seroprevalence reports from Mozambique, Zimbabwe, Tanzania and South Africa ranged between 1–21.3% in rodents and 4.1–51.2% in humans.\(^{44}\) A comprehensive review listed seroprevalences of 4–100% for women of child-bearing age in countries across the globe between 1990 and 2000.\(^{53}\) Humans can contract *T. gondii* by eating raw or undercooked flesh of various intermediate hosts, therefore human infections of *T. gondii* are not always related to rat infections.\(^{53}\)

Age of rodents was the only significant predictor for *M. moniliformis* prevalence (9.5%), as pups are much less likely to be infected than adults due to the five-week prepatent period of the parasite. This “thorny-headed worm” was described from *R. rattus* and *R. norvegicus* in Egypt\(^{44}\) yet is rare in southern Africa. Prevalence of *M. moniliformis* in the gut of *Rattus* spp. from across the globe range between 0% and 59.3%.\(^{7}\) There have been clinical case reports of *M. moniliformis* infections in people from Australia, Asia, Europe, America and Africa (Sudan, Nigeria, Egypt, Madagascar and Zimbabwe).\(^{5,6}\) In Ghana, *West Africa*, larval stages of *M. moniliformis* were found in the intermediate host, *Periplaneta americana*.\(^{57}\) There is evidence that worm abundance is positively correlated with abundance of common arthropod intermediate hosts, particularly in the hot, humid rainy season.\(^{58}\) Although we found no evidence for the influence of season on prevalence, worm abundance was significantly high during the wet season at harbour and informal settlement sites.

*Caldium hepaticum* was present in male and female *R. norvegicus* in both seasons at both the harbour (*n = 3*) and urban/peri-urban (*n = 7*), and one *M. natalensis* (U/PUR, dry season) had a severe liver infection. Transmission of parasite eggs is facilitated mainly by cannibalism and necrophagy, particularly inside burrows; and, once eggs mature to the infective stage, allogrooming can cause re-infection.\(^{59,60}\) We found no evidence that prevalence and intensity were related to season or gender of the host. Low prevalence of *C. hepaticum* may be due to the relatively mild winters and abundant food resources throughout the year in Durban, which, in turn, may result in low levels of cannibalism and necrophagy. Human hepatic capillaritis is a serious infection that is usually diagnosed at autopsy, yet there were only two clinical cases and one autopsy reported from South Africa between 1957 and 1973.\(^{51,61,62}\)

*Hymenolepis nana* was found only in three rats at the harbour in the wet season. This tapeworm needs only one host to complete its lifecycle.\(^{63}\) Eggs are infective when they leave the gravid segments and can infect the same host by anus-hand-mouth contamination or via oncospheres hatching from eggs laid in the gut, or from another host via eggs in contaminated food. Previous studies have estimated *H. nana* prevalence in *R. norvegicus* between 0% and 42.4%\(^{64,65}\); reports have been varied in humans. For example, low prevalences in school-based surveys where infected children were symptomless\(^{66,67}\) and individual symptomatic cases in children from disadvantaged backgrounds as a concomitant infection with *Giardia intestinalis*,\(^{68}\) or in immunocompromised patients together with *Cryptosporidium parvum*.\(^{69}\)

The most common mechanically transmitted eggs carried by *R. norvegicus* in this study belonged to *Ascaris* sp. (probably *A. lumbricoides*). Infected rats were caught, mainly at urban/peri-
urban sites; almost half of the carrier rats were caught under a wooden hut that was used as a creche. Previously, children in informal settlements of eThekwini exhibited high prevalence of A. lumbricoides (81.7–96.3%). It is perhaps notable that 39% of boys and 53% of girls in northern KwaZulu-Natal regularly ate soil.

Conclusions

All eight parasites identified in this study are of public health importance and capable of causing pathology, with C. hepaticum the most serious and H. diminuta the least. Although prevalences of parasites were relatively low, the mere presence of these parasites merits further investigation in eThekwini as well as other metropolitan areas in Africa. We found evidence that location, season, rat age and gender differentially affected the prevalence and mean worm abundance of parasite species. However, one caveat of this study is that it was not possible to design the sampling protocol ourselves, especially in the slums, partly because of the high crime rates. Thus sampling effort was not consistent and equal across locations and seasons, which may have affected our statistical analyses of parasite prevalence and abundance. Future work should standardise the sampling effort and replicate sampling in other urban landscapes, to better understand the patterns and drivers of parasite loads in rats in African urban landscapes.

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Conflict of Interest – The authors declare that they do not have a commercial or any other association which might pose a conflict of interest.

References

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