



Rodent-borne parasites in Qatar: A possible risk at the human-animal-ecosystem interface

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ABSTRACT

Rodents are known reservoirs for a diverse group of zoonotic pathogens that can pose a threat to human health. Therefore, it is crucial to investigate these pathogens to institute prevention and control measures. To achieve this, the current study was conducted to investigate the frequency of different parasites in commensal rodents in Qatar. A total of 148 rodents, including *Rattus norvegicus*, *Rattus rattus*, and *Mus musculus* were captured using traps placed in different habitats such as agricultural and livestock farms, residential areas, and other localities. Blood, feces, ectoparasite, and visceral organs were collected for gross, microscopic, immunological, and molecular analysis. The study identified 10 different parasites, including *Capillaria annulosa*, *Eimeria* spp., *Giardia* spp., *Hymenolepis diminuta*, *Mastophorus muris*, *Ornithonyssus bacoti*, *Taenia taeniaeformis*, *Toxoplasma gondii*, *Trypanosoma lewisi*, and *Xenopsylla astia*. Overall, 62.2% of the rodents tested positive for at least one parasite species. Helminths were found to be the most prevalent parasites (46.0%), followed by ectoparasites (31.8%), and protozoa (10.1%). However, individually, *X. astia* was the most prevalent (31.8%), whereas *C. annulosa* was the least common (0.7%). The prevalence of *X. astia* and *H. diminuta* significantly differed between habitats ($p < 0.05$). The sequence analysis of *Hymenolepis* spp. was closely related to the previously reported *H. diminuta* in Iran, China, and Mexico. In conclusion, the study identified a diverse range of rodent-borne parasites that are important to public health, with most of them being recorded for the first time among commensal rodents in Qatar.

1. Background

The majority of emerging and re-emerging infectious diseases are of zoonotic origin [1] and viral in nature [2]. Many countries are facing a

rising number of parasitic infections as well [3]. The spillover of zoonotic parasites to the humans occurs from different sources, including livestock, pets, poultry, fishes, and wild animals, including rodents [4,5]. Rodents are the largest terrestrial mammalian group, with

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approximately 10% are considered pests [6]. In Middle Eastern countries, an estimated 100 helminth species have been reported in rodents, of which 22 are of considerable public health concern [7]. These animals also carry several vectors for zoonotic pathogens. For instance, the oriental rat flea, *Xenopsylla cheopis* carries *Rickettsia typhi*, the causal agent of typhus fevers in humans [8]. Some rodent-borne diseases, such as Q fever, leishmaniasis, schistosomiasis, and soil-transmitted helminthosis, are considered to be neglected tropical diseases, hence little attention has been paid to their prevention and control. As a result, they are detrimental to human and animal health, as well as economic sustainability, due to increasing morbidity, mortality, production loss, and medical costs [9,10]. Recently, the burden of Q fever was reported to be far greater among humans than in animals [11].

Qatar is a small desert country located in the Arabian Peninsula with three predominant species of commensal rodents: *Rattus norvegicus*, *Rattus rattus* and *Mus musculus*, [12,13]. The country has experienced rapid urbanization and population growth over the last few decades [14–16]. Several rodent-borne parasitic diseases, including echinococcosis, hymenolepiasis, schistosomiasis, cryptosporidiosis, giardiasis, leishmaniasis, and toxoplasmosis, have been reported in this country [17–22]. Currently, there is a significant knowledge gap regarding zoonotic transmissions and dynamics of these parasites in Qatar [23]. Population demographics, rapid urbanization, and agricultural and livestock ventures are all conducive to rodent-mediated parasitic transmission between species at the human-animal-ecosystem interface [3,9,24], with rodents facilitating parasite transmission across these factors. Understanding a disease, its possible hosts, risk factors, and transmission dynamics is essential for early preparedness, prevention, and control [25]. Therefore, the aim of this study was to determine the frequency of parasites in commensal rodents in different habitats in Qatar, as well as identify the factors associated with their occurrence.

2. Materials and methods

2.1. Rodent collection and identification

A total of 148 rodents, 120 *R. norvegicus*, 24 *R. rattus*, and 4 *M. musculus*, were captured using 250 baited traps, consisting of 100 multi rodent traps (MRT) and 150 single rodent traps (SRT) between August 2019 and February 2020. The traps were placed in the evening and left overnight in different habitats across eight municipalities of Qatar, including agricultural and livestock farms, human residence, and commercial and industrial areas (Fig. 1). The successful traps were collected the next day morning and transported to the Qatar Central Veterinary Laboratory at the Ministry of Municipality. The rodents were examined within four hours after their arrival at the laboratory [13]. The location of capture, habitat, species, age, sex, trap type, body mass index (BMI), and pregnancy status of the trapped rodents were recorded. The BMI was calculated by dividing the body weight (in grams) by the square of the length (in centimeters) (i.e., $BMI = \text{body weight (g)} / \text{length}^2 \text{ (cm}^2\text{)}$) [26].

2.2. Blood microscopic examination and ELISA

The rodents were anesthetized using 5% isoflurane inhalation for 3–5 min. A volume of 3–5 ml of blood was collected via cardiac puncture using a vacutainer EDTA tube [27]. The collected blood samples were subsequently examined under a microscope with Giemsa staining for parasites [28]. Commercial ELISA test kits, the multi-species ID Screen® Toxoplasmosis IgG Indirect kit (IDVET, Montpellier) and the *Leishmania* IgG kit® (Cat No. IB0510, IBL, Minneapolis), were used to detect the presence of anti *T. gondii* and *Leishmania* spp. IgG antibodies, following the manufacturers instruction.

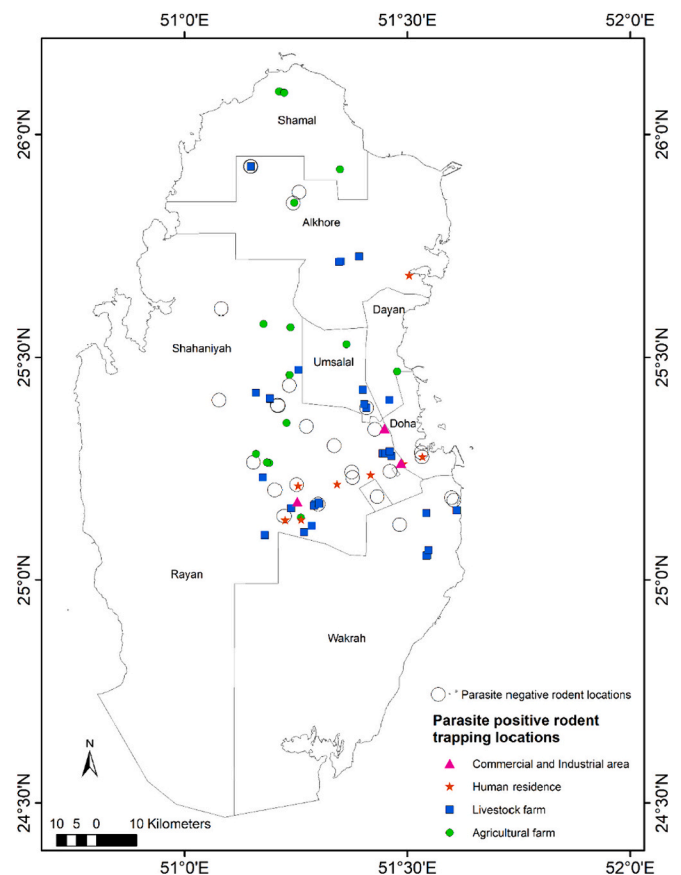


Fig. 1. Location of the trapped rodents in different municipalities of Qatar.

2.3. Ectoparasite collection and identification

Ectoparasites were collected from the rodents using a hairdryer, as described elsewhere [29]. The area of the ears, face, and perineum were thoroughly examined for the presence of mites and ticks [30]. The ectoparasites found on each rodent were counted and recorded. Direct microscopy was used to validate their identity using conventional external identification criteria [31–33].

2.4. Necropsy, feces examination, and histopathology

Following euthanasia, the rodents underwent necropsy, and their liver, stomach, and intestine were examined for the presence of parasitic cysts, worms, or eggs, using conventional identification criteria [34–36]. Additionally, six visceral samples, including the liver, lungs, spleen, kidney, intestine, and diaphragm, were aseptically collected from each rodent and stored at $-40\text{ }^{\circ}\text{C}$ for further analysis. Fecal samples were examined for parasitic eggs or ova using microscopy and direct smear techniques with normal saline [36–40]. Furthermore, a small piece of liver from each rodent was fixed in 10% neutral buffered formalin and subjected to paraffin-embedded histological analysis. The liver blocks were sectioned at a $5\text{ }\mu\text{m}$ diameter using a microtome, stained with routine hematoxylin and eosin stains [41], and examined under a microscope for *Capillaria hepatica*.

2.5. Molecular assays

2.5.1. Sample preparation and genomic DNA extraction

The visceral specimens obtained from each rodent were pooled and homogenized to form a single tissue pool [42]. Genomic DNA was extracted from fecal samples using the QIAamp DNA stool mini kit®, while tissue pools and five randomly selected cestodes were processed

using the QIAamp Tissue Mini Kit®, according to manufacturer's instructions (Qiagen, CA, USA). The extracted genomic DNA was stored at -80 °C for further use.

2.5.2. PCR based identification

Conventional and nested PCR techniques were performed to detect *Hymenolepis* spp. and *Giardia lamblia*, respectively. Real-time PCR was used for the detection of *Leishmania* spp. and *T. gondii*. The details of the samples used for pathogen detection, PCR reaction mixture, primers/probes, and amplification are provided in Table 1. *ITS 1* and *5.8 s rRNA*, *gdh*, *aap3*, and *b1* genes were included as a positive controls in each amplification reactions. The products of conventional and nested PCR were separated by electrophoresis in a 1% agarose gel (Sigma-Aldrich, USA) and visualized under a UV transilluminator after staining with 0.5 µg/ml of ethidium bromide.

2.5.3. Sequencing and phylogenetic analysis

The PCR products of cestode samples were purified using the Add-Prep PCR purification kit according to the manufacturer's instructions (www.addbioinc.com). Sanger sequencing was performed on the purified products at SolGent Co. Ltd., Daejeon, 34,014, Korea (<http://www.solgent.com>). The sequencing electropherograms were analyzed using the BioEdit Sequence Alignment Editor, and sequence alignment was generated using Gene Doc software. BLAST similarity searches were conducted in the GeneBank database, and representative sequences from other regions of the world were downloaded for comparative analysis. Furthermore, all sequences generated in this study were compared with each other within their respective groups to calculate percent pairwise identities. To study the genetic relationship of *H. diminuta* among global sequence, phylogenetic analysis was performed using MEGA-11 software (<https://www.megasoftware.net/>). Hasegawa-Kishino-Yano (HKY) model with 1000 bootstrap replications was used for constructing a Neighbour-Joining tree in Geneious Prime 2022.0.2 software.

2.6. Statistical analysis

The field and laboratory data were recorded in a Microsoft Excel 2016 spreadsheet, and statistical analyses were performed using STATA/IC- 13 (STATA Corp LLC, Lakeway Drive, TX, USA). Descriptive analysis was performed to calculate the overall prevalence of parasites, which was expressed as a percentage along with the frequency and a 95% confidence interval (CI). Ectoparasite indices were determined for each type of ectoparasite (fleas or mites) by dividing the total number of detected ectoparasites by the total number of rodents sampled [47].

Univariate analysis was conducted using the Fisher exact test to

explore associations between parasitic prevalence and various factors related to demographics (rodent species, age, sex, and BMI), trap type, and habitats. Univariate logistic regression was performed to assess the strength of association between the two specific parasites and the fleas, which were assumed to be the intermediate host for those parasites. The results of the univariate logistic regression were presented as percentages and *p*-values.

3. Results

3.1. Description and general prevalence of parasites

A total of 10 parasite species were identified in the tested rodents (Table 2). Helminths were found to be the most common parasite (45.9%), followed by ectoparasites (31.8%) and protozoa (10.1%). Regarding individual species, *X. astia* was the most prevalent (31.76%) parasite, whereas the least prevalent parasite was *Capillaria annulosa* (0.68%).

The overall parasitic prevalence was 62.2% (Table 2), with 31.8% positive for one parasite species, and 30.4% were coinfecting (carrying two or more parasite species) (Table 3). As the capture number of *R. rattus* (*n* = 24) and *M. musculus* (*n* = 4) was small, no further statistical analysis for these two species could be performed.

3.2. Rodent-borne ectoparasites

On the trapped rodents, 249 fleas (*X. astia*) were found, with 79% being female and 21% male. All three rodent species had *X. astia*, with a prevalence of 35.0% on *R. norvegicus*, 16.7% on *R. rattus*, and 25.0% on *M. musculus*. This corresponds to flea indices of 1.9, 0.6, and 0.8, respectively. Additionally, four mites (*Ornithonyssus bacoti*) were detected only on *R. norvegicus*, with a mite prevalence of 3.3% and an index of 0.03. The Fisher exact test revealed a significantly higher prevalence of *X. astia* (*p* = 0.01) on *R. norvegicus* from agricultural farms (48.3%), followed by livestock farms (39.7%), and other sources (15.2%) (Table 4).

3.3. Rodent-borne helminths

H. diminuta was the most prevalent helminth (28.4%) detected in the intestine and feces (Fig. 2 and Table 2) and was confirmed by PCR followed by Sanger sequencing and phylogenetic analysis. The phylogenetic analysis revealed that the five cestodes shared two identical gene sequences, assigned NCBI GenBank accession numbers OM778284 and OM773635. These two gene sequences showed 97–100% nucleotide sequence identity with the *H. diminuta* reference genomes. The

Table 1

Primers, probes, and the PCR conditions for detecting *Hymenolepis* spp., *Giardia lamblia*, *Leishmania* spp., and *Toxoplasma gondii* in this study.

Pathogen and sample	Primer name	Primer (5'-3')	PCR conditions	Reference
<i>Hymenolepis</i> spp.; feces and cestode	Hym spF	GCGGAAGGATCATTACACGTTTC	95 °C for 10 min, 35 cycles of 95 °C for 30 s, 60 °C for 30 s and 72 °C for 30 s, 72 °C for 5 min, and 12 °C hold	[43]
	Hym spR	GCTGCACTCTTCATCGATCCAGG		
<i>Giardia lamblia</i> ; feces	GDHeF	TCAAACGTAAAYCGYGGYTTTC	95 °C for 10 min, 35 cycles of 95 °C for 30 s, 60 °C for 30 s and 72 °C for 30 s, 72 °C for 5 min, and 12 °C hold	[44]
	GDHiF	CAGTACAACCTCYGCTCTCGG		
	GDHiR	GTTRTCCTTGACATCTCC		
<i>Leishmania</i> spp.; tissue	LeishF	GGCGGC-GGTATTATCTCGAT	50 °C for 2 min, 95 °C for 10 min, 40 cycles of 95 °C for 15 s, 60 °C for 1 min, and 10 °C hold	[45]
	LeishR	ACCACGAGGTAGATGACAGACA		
	Leish (Probe)	FAM-ATGTCGGGCATCATC-NFQ		
<i>Toxoplasma gondii</i> ; tissue	ToxoF	TCCCCTCTGCTGGCGAAAAGT	45 °C for 10 min, 95 °C for 10 min, 45 cycles of 95 °C for 15 s, 60 °C for 45 s and 10 °C hold	[46]
	ToxoR	AGCGTTCGTGGTCAACTATCG		
	Toxo (Probe)	FAM-TCTGTGCAACTTTGGTGT		
		ATTCGCAG-TAMRA		

Table 2
Commensal rodent-borne parasitic prevalence in Qatar.

Parasites	<i>Rattus norvegicus</i> (n = 120)	<i>Rattus rattus</i> (n = 24)	<i>Mus musculus</i> (n = 4)	Overall (N = 148)
Ectoparasites	42, 35.0 (26.5–44.2)	4, 16.7 (4.8–37.4)	1, 25.0 (0.6–80.6)	47, 31.8 (24.4–39.9)
<i>Ornithoryssus bacoti</i>	4, 3.3 (0.9–8.3)	–	–	4, 2.7 (0.7–6.8)
<i>Xenopsylla astia</i>	42, 35.0 (26.5–44.2)	4, 16.7 (4.8–37.4)	1, 25.0 (0.6–80.6)	47, 31.8 (24.4–39.9)
Helminths	61, 50.8 (42.0–59.6)	6, 25.0 (9.8–46.7)	1, 25.0 (0.6–80.6)	68, 45.9 (38.1–54.0)
<i>Capillaria annulosa</i>	1, 0.8 (0.12–4.6)	–	–	1, 0.7 (0.1–3.7)
<i>Hymenolepis diminuta</i>	36, 30.0 (21.9–39.1)	6, 25.0 (9.8–46.7)	–	42, 28.4 (21.3–36.4)
<i>Mastophorus muris</i>	23, 19.2 (12.6–27.4)	–	1, 25.0 (0.6–80.6)	24, 16.2 (10.7–23.2)
<i>Taenia taeniaeformis</i>	25, 29.1 (19.8–39.1)	–	–	25, 16.9 (11.7–23.7)
Protozoa	9, 7.5 (4.0–13.6)	6, 25 (12.0–44.9)	–	15, 10.1 (6.2–16.0)
<i>Eimeria</i> spp.	4, 3.3 (1.3–8.3)	1, 4.1 (0.7–20.2)	–	5, 2.7 (1.1–6.7)
<i>Giardia</i> spp.	4, 3.3 (0.9–8.3)	2, 8.3 (1.1–26.3)	–	6, 4.1 (1.5–8.6)
<i>Toxoplasma gondii</i>	2, 2.3 (0.3–8.2)	–	–	2, 1.4 (1.3–5.3)
<i>Trypanosoma lewisi</i>	1, 0.8 (0.12–4.6)	3, 12.5 (2.7–32.3)	–	4, 2.7 (0.7–6.8)
Overall	77, 64.2 (55.3–72.2)	13, 54.2 (35.1–72.1)	2, 50.0 (15.0–85.0)	92, 62.2 (54.1–69.6)

Result presented as total number of positive rodents, prevalence (95% Confidence Interval)

Table 3
Parasite species load among different rodent species.

Rodent species	No of parasite species	Total rodent positive, % (95% CI)
<i>Rattus norvegicus</i> (n = 120)	1	35, 29.2 (21.8–37.8)
	2	27, 22.5 (15.9–30.8)
	3	10, 8.3 (4.6–14.7)
	4	3, 2.5 (0.9–7.1)
	5	1, 0.9, (0.1–4.6)
	6	1, 0.9, (0.1–4.6)
<i>Rattus rattus</i> (n = 24)	1	10, 41.7 (24.5–61.2)
	2	3, 12.5 (4.3–31.0)
<i>Mus musculus</i> (n = 4)	1	2, 50.0 (15.0–85.0)

Neighbour-Joining phylogenetic tree placed them in the same clade, closely related to sequences reported from the Canary Islands, South Africa, Australia, Iran, Spain, China, and Mexico (Fig. 3).

Table 4
Univariate association between different categories and ectoparasite prevalence in *Rattus norvegicus*.

Categories	<i>Xenopsylla astia</i>		<i>Ornithoryssus bacoti</i>	
	Positive (%)	p-value	Positive (%)	p-value
Age				
Adult (n = 115)	41 (35.7)	0.47	4 (3.5)	0.67
Young (n = 5)	1 (20.0)		0 (0.0)	
Sex				
Female (n = 62)	21 (33.9)	0.79	3 (4.8)	0.34
Male (n = 58)	25 (36.2)		1 (1.7)	
Pregnancy				
Pregnant (n = 16)	4 (25.0)	0.38	1 (6.3)	0.76
Non-pregnant (n = 46)	17 (37.0)		2 (4.4)	
Body Mass Index				
Low (13)	4 (30.8)	0.75	0 (0.0)	0.42
Normal (67)	26 (38.8)		4 (6.0)	
High (35)	11 (31.4)		0 (0.0)	
Habitats				
Agricultural farm (n = 29)	14 (48.3)	0.01	1 (3.5)	0.42
Livestock farm (n = 58)	23 (39.7)		3 (8.2)	
Other areas* (n = 44)	5 (15.2)		0 (0.0)	
Trap type				
Single rodent trap (51)	19 (37.3)	0.70	1 (2.0)	0.64
Multi rodent trap (69)	23 (33.3)		3 (4.3)	

* Other areas: Human residence, industrial area, and commercial area.

Furthermore, 24 rodents (16.2%) were tested positive for *Mastophorus muris*, either through detection of nematodes in the stomach or eggs in the feces (Fig. 2). Notably, none of the *R. rattus* tested positive for *M. muris*. The majority (70%) of the 25 livers positive for *Cysticercus fasciolaris* (Cysticerci of *Taenia taeniaeformis*) had a single cyst, whereas the remaining livers (30%) had two to three cysts. *T. taeniaeformis* and *C. annulosa* were only detected in *R. norvegicus*. However, neither gross nor histological investigation revealed the presence of *C. hepatica* in the livers.

The Fisher exact test revealed that the prevalence of *H. diminuta* was significantly higher ($p = 0.00$) in the livestock farms (39.7%), followed by agriculture farms (37.9%) and other sources (6.1%) (Table 5). The logistic regression revealed a positive correlation between the prevalence of *H. diminuta* (OR = 4.13; $p = 0.00$) and the higher prevalence of *X. astia* (Table 6).

3.4. Rodent-borne protozoa

Out of the 148 tested fecal samples, five samples were positive for *Eimeria* spp. (Fig. 4). Six fecal specimens were found to be positive for *Giardia* spp. by microscopic examination, but negative for *Giardia lamblia* by PCR. By ELISA or PCR, none of the samples tested positive for *Leishmania* spp. Two samples (1.4%) tested positive for IgG against *T. gondii* by ELISA, however none of the tissue samples were positive by PCR. It is worth noting that *Eimeria* spp. exhibited a higher prevalence among female rodents (4.8%) compared to male rodents (1.7%) ($p = 0.03$) in *R. norvegicus* (Table 7). Additionally, *Giardia* spp. showed a significantly higher prevalence ($p = 0.01$) among young rodents (40.0%) compared to adults (1.7%).

4. Discussion

Most emerging infectious diseases have their origins in wildlife species, and changes in the human-animal-ecosystem interface play a significant role in facilitating pathogen transmission at this interface. While many parasites do not cause the death of their host, they can induce various pathologies, including weakness and anemia. Parasitic infection in rodents can reduce their competitive abilities and make them more susceptible to predators [48,49]. However, certain rodent-borne parasites such as leishmaniasis, toxoplasmosis, and hymenolepiasis are extremely important at the One Health interface. The goal of this study was to assess the risk of rodent-borne parasites at the human-animal interface and determine the frequency patterns across different habitats in Qatar. A total of ten parasite species were identified in this study, of which only *X. astia* and *H. diminuta* had been previously reported [50]. The remaining eight parasite species were detected in the country for the first time.

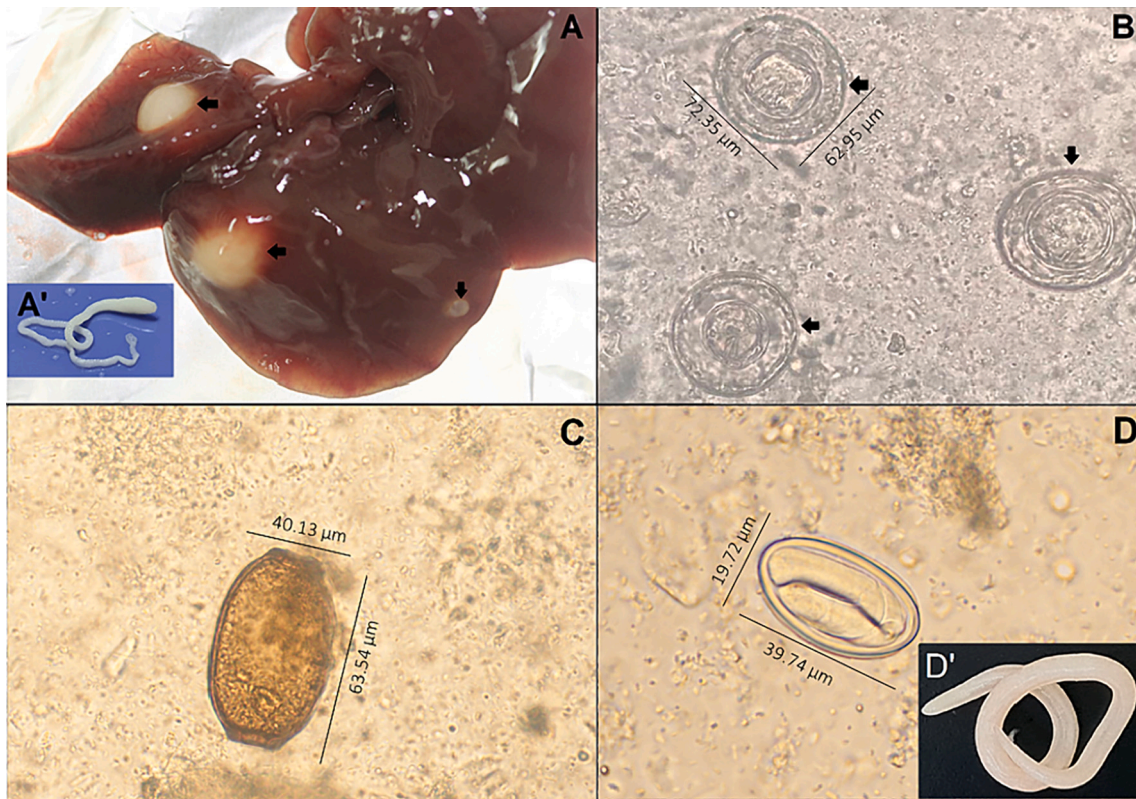


Fig. 2. Helminths detected from commensal rodents in Qatar. *Cysticercus fasciolaris* (A' is the larva from a cyst) in a liver (A), Eggs of *Hymenolepis diminuta* (B), *Capillaria annulosa* (C), and *Mastophorus muris* (D' is a *M. muris* found in rodent stomach) (D) in rodent feces.



Fig. 3. The Neighbour-Joining phylogenetic tree of the *Hymenolepis diminuta* ITS1 and 5.8 s ribosomal RNA partial gene sequences generated in the present study from rodents in Qatar ($n = 2$) and related sequences downloaded from NCBI-GenBank reported during 2003–2022 worldwide.

Table 5
Univariate association between different categories and helminth prevalence in *Rattus norvegicus*.

Categories	<i>Hymenolepis diminuta</i>		<i>Mastophorus muris</i>		<i>Taenia taeniaeformis</i>	
	n (Positive) %	p-value	n (Positive) %	p-value	n (Positive) %	p-value
Age						
Adult	115 (36) 31.3	0.14	115 (23) 20.0	0.27	82 (25) 30.5	0.19
Young	5 (0) 0.0		5 (0) 0.00		4 (0) 0.00	
Sex						
Female	62 (18) 29.0	0.81	62 (14) 22.6	0.33	38 (8) 21.1	0.15
Male	58 (20) 31.0		58 (9) 15.5		48 (17) 35.4	
Pregnancy						
Pregnant	16 (6) 37.5	0.39	16 (2) 12.5	0.26	11 (2) 18.2	0.78
Non-pregnant	46 (12) 16.1		46 (12) 26.1		27 (6) 22.2	
Body Mass Index						
Low	13 (2) 15.4	0.34	13 (2) 15.4	0.49	10 (2) 20	0.34
Normal	67 (21) 31.3		67 (16) 23.9		50 (18) 36.0	
High	35 (13) 37.1		35 (5) 14.3		5 (5) 25.0	
Habitats						
Agricultural farm	29 (11) 37.9	0.00	29 (5) 17.2	0.67	24 (9) 37.5	0.22
Livestock farm	58 (23) 39.7		58 (13) 22.4		46 (14) 30.4	
Other areas*	33 (2) 6.1		33 (5) 15.1		16 (2) 12.5	
Trap type						
Single rodent trap	51 (21) 41.2	0.03	51 (10) 19.6	1.00	40 (8) 20.0	0.26
Multi rodent trap	69 (15) 21.7		69 (13) 18.8		46 (17) 11.6	

* Other areas: Human residence, industrial area, and commercial area.

Table 6
Univariate logistic regression of the effect of fleas on the prevalence of parasites in rodents.

Parasites	Odds Ratio	p-value	95%CI
<i>Hymenolepis diminua</i>	4.13	0.00	1.93–8.83
<i>Trypanosoma lewisi</i>	0.73	0.79	0.06–7.98

In the present study, we found that the prevalence of the flea was highest on *R. norvegicus*, followed by *R. rattus* and *M. musculus*. This finding is inconsistent with a previous report in the Middle East, where the overall prevalence of fleas was highest on *R. norvegicus* (44.4%), followed by *R. rattus* (33.9%), and *M. musculus* (21.6%) [51]. Recently, we reported that rodent-borne fleas and mites in Qatar carry *Rickettsia* spp. [42], suggesting that *X. astia* and *O. bacoti* may play a role in transmitting Rickettsial pathogens at the human-animal-ecosystem interface through rodents [23].

Among the four helminth species identified in this study, *H. diminuta* and *T. taeniaeformis* were the most prevalent. *H. diminuta* is a common intestinal parasite found in small rodents worldwide [7,52], including in Qatar [50]. *H. diminuta* can be transmitted by arthropod vectors such as beetles, caterpillars, cockroaches, and fleas [52]. Humans, particularly children, can acquire *H. diminuta* infection by accidentally ingesting the intermediate host containing the cysticerci of the cestode [52,53]. A previous study identified cysticerci of *H. diminuta* in *X. cheopis* [54]. In this study, a positive correlation was observed between the prevalence of *H. diminuta* and *X. astia*, which corroborates with previous studies [50]. Cats are the primary host and rodents act as intermediate host of *T. taeniaeformis* [36]. Previous studies detected eggs of *T. taeniaeformis* in cats in Qatar [17], indicating that it is a common parasite at the cat-rodent interface in the country. Although *M. muris* and *C. annulosa* are not considered of significant public health concern [7], they can negatively impact rodent health [55]. Previous studies have reported the presence of *M. muris* in rodents in Egypt and Iran [56,57], and *C. annulosa* in Iran [58].

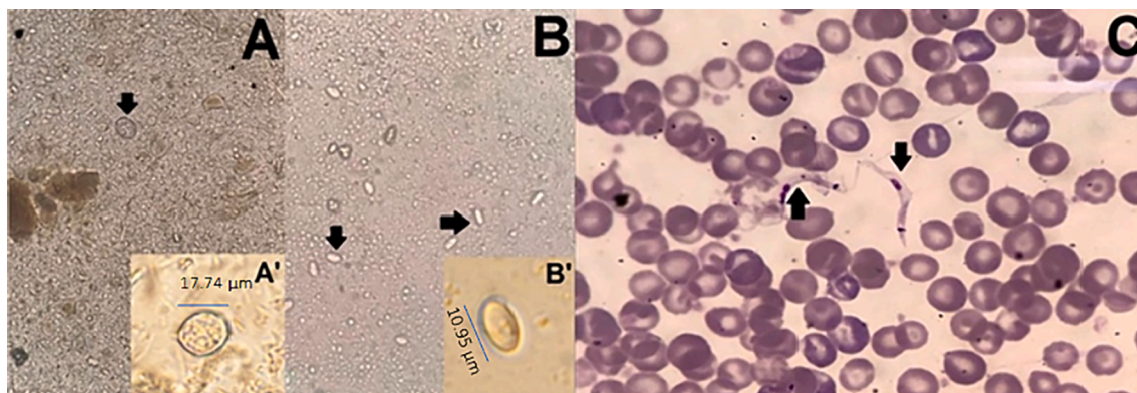


Fig. 4. *Eimeria* spp. oocyst (A) and *Giardia* spp. oocyst (B) in feces and *Trypanosoma lewisi* worm (C) in blood (indicated by arrows) of rodents in Qatar. A' and B' are the large size figure of the respective parasite oocysts.

Table 7
Univariate association between different categories and protozoa prevalence in *Rattus norvegicus*.

Categories (n)	<i>Eimeria</i> spp.		<i>Giardia</i> spp.		<i>Toxoplasma gondii</i>	
	Positive (%)	p-value	Positive (%)	p-value	Positive (%)	p-value
Age						
Adult (115)	4 (3.5)	1.00	2 (1.7)	0.00	2 (1.7)	0.77
Young (5)	0 (0.0)		2 (40.0)		0 (0.0)	
Sex						
Female (62)	3 (4.8)	0.03	1 (1.6)	0.28	0 (0.0)	0.14
Male (58)	1 (1.7)		3 (5.2)		2 (3.45)	
Pregnancy						
Pregnant (16)	3 (18.8)	1.00	0 (0.0)	0.55	0 (0.0)	–
Non-pregnant (46)	0 (0.0)		1 (2.2)		0 (0.0)	
Body Mass Index						
Low (13)	1 (7.7)	0.07	0 (0.0)	0.64	0 (0.0)	0.64
Normal (67)	2 (3.0)		2 (3.0)		2 (3.0)	
High (35)	1 (2.9)		0 (0.0)		0 (0.0)	
Habitats						
Agricultural farm (29)	2 (6.7)	1.00	1 (3.5)	1.00	0 (0.0)	0.34
Livestock farm (58)	0 (0.0)		2 (3.5)		2 (3.45)	
Other areas* (22)	2 (9.1)		1 (3.0)		0 (0.0)	
Trap types						
Single rodent trap (51)	1 (2.0)	0.64	2 (3.9)	1.00	0 (0.0)	0.51
Multi rodent trap (69)	3 (4.5)		2 (2.9)		2 (2.3)	

* Other areas: Human residence, industrial area, and commercial area.

Eimeria parasites are not zoonotic but are economically important as they infect livestock and poultry [59]. However, the degree of host specificity of rodent eimeriosis is still not clearly known [60]. *Giardia muris* and *Giardia microti* are commonly reported among rodents but do not have any zoonotic importance [61]. On the other hand, *G. lamblia* is a zoonotic parasite that can infect humans, pets, livestock, and other mammals, including rodents [62]. In Qatar, *G. lamblia* is a common cause of enteritis among humans and animals [22,63]. The *Girardia* spp. detected in this study are not *G. lamblia*; they may be some other species, like *G. muris* and *G. microti*, which requires further confirmation.

A single study reported that toxoplasmosis is endemic among humans in Qatar [20]. This protozoan has been reported to cause abortion among livestock animals in this country [63]. Cats are considered a source of disease transmission at the human-animal interface [18,64]. In the present study, *T. gondii* seroprevalence was found to be low in rodents. Furthermore, *T. lewisi* causes murine trypanosomiasis in domestic rodents worldwide [32] and occasionally infects humans [65,66]. Previous studies have reported that *Ceratophyllus fasciatus*, *Nosopsyllus fasciatus*, *X. cheopis*, and *Xenopsylla nubica* act as biological carriers of *T. lewisi* [67,68], but there is a dearth of knowledge on the pathophysiology and epidemiology of *T. lewisi* in Qatar due to lack of studies.

The current surge in emerging and re-emerging infectious diseases has aroused public health concerns for medical and veterinary practitioners as well as policymakers regarding their origin, transmission dynamics, and severity [2,9]. We identified ten species of parasites that infect rodents in Qatar, some of which have the potential to infect humans, livestock, dogs, cats and other animals. Peri-urban rodents, such as *Rattus* spp. and *Mus* spp. usually prefer to live in cities [69].

Urbanization can increase the likelihood of rodent infestation and risk of related zoonoses at the human-animal interface [48,70,71]. This is important in light of the rapid urbanization and agricultural development in Qatar over recent years. *X. astia* and *H. diminuta* across different habitats in Qatar, with higher prevalence in agricultural and animal farming facilities. The animal farms in Qatar typically keep multiple species of animals with minimal biosecurity practices, which makes it easier for rodents to infest these places. For this region, it is commonly and strongly recommended to institute well-structured and enhanced farm biosecurity measures to control the spread of identified parasites and their zoonotic transmission at the human-animal-ecosystem interface. It is crucial to comprehend the burden of the rodent population and maintain an eco-friendly rodent population within the Qatari ecosystem through integrated pest management [72]. Public health authorities, community leaders, and policymakers should establish guidelines and policies aimed at diminishing rodent-borne zoonotic risks. A shared awareness within the community and support from the media are pivotal aspects in achieving this objective [2]. A One Health framework was proposed to combat the risks associated to rodent-borne pathogens in Qatar [73]. We suggest to enrich and implicate the framework as presented in Fig. 5.

Our study has some limitations: first, due to limited resources, direct smear method was used to detect fecal parasites, which may have underestimated parasitic prevalence due to false-negative results; second, we collected a limited number of samples from *M. musculus* and *R. rattus*; third, the use of MRT could have led to biased ectoparasite prevalence.

5. Conclusions

The current study has identified a diverse range of rodent-borne ectoparasites, helminths, and protozoa. Out of the 10 species of rodent-borne parasites, *X. astia*, *O. bacoti*, *H. diminuta*, *T. gondii*, *T. lewisi*, and *T. taeniaeformis* are important for public health and, as such, require urgent action for their prevention and control. Commensal rodents can mediate the transmission of these parasites at the human-animal-ecosystem interface. In addition, with the exception of *X. astia* and *H. diminuta*, all parasite species detected in this study are for the first time recorded in commensal rodents across different habitats in the

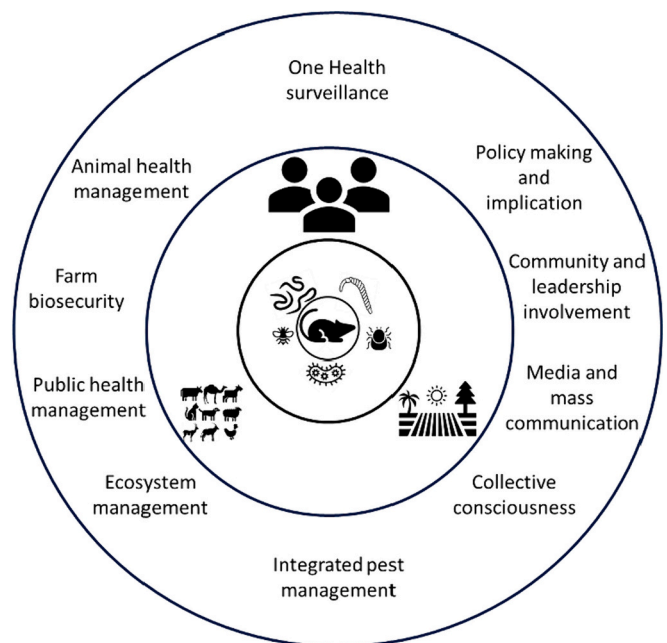


Fig. 5. Possible key activities to combat risk associated with rodent-borne parasites for effective One Health intervention.

history of Qatar. Further studies are required to identify the biology and transmission dynamics of these parasites in these habitats.

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Ethical permission

Ethical clearance for the current study was obtained from the “Institutional Animal Care and use Committee” of the Ministry of Municipality and Environment in the State of Qatar. with permission number IACUC-A-MME-4, dated 09 Feb 2020.

Authorship contribution statement

Md Mazharul Islam: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Elmoubashar Farag:** Conceptualization, Resources, Supervision, Visualization, Writing – review & editing. **Mohammad Mahmudul Hassan:** Formal analysis, Writing – original draft, Writing – review & editing. **Khalid A. Enan:** Methodology, Writing – review & editing. **Ali Mohammadi:** Methodology, Writing – review & editing. **Amneh Khaleel Aldiqs:** Methodology, Writing – review & editing. **Hashim Alhussain:** Methodology, Writing – review & editing. **Ebtesam Al Musalmani:** Investigation, Methodology, Writing – review & editing. **Abdul Azia Al-Zeyara:** Project administration, Writing – review & editing. **Hamad Al-Romaihi:** Funding acquisition, Project administration, Writing – review & editing. **Hadi M. Yassine:** Resources, Writing – review & editing. **Ali A. Sultan:** Funding acquisition, Visualization, Writing – review & editing. **Devendra Bansal:** Visualization, Writing – review & editing. **Zilungile Mkhize-Kwitshana:** Conceptualization, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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