

## ORIGINAL RESEARCH

# Zoonotic risks in urban areas: Prevalence of helminth parasites in urban populations of *Lissachatina fulica* in Sarawak, Malaysia

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## Abstract

**Background:** The giant African land snail (*Lissachatina fulica*), an invasive species with significant medical, agricultural and economic importance, is abundant in urban areas of Kota Samarahan, Sarawak. The proximity between humans and the snail may increase the risk of pathogen transmission. However, no study has examined the helminth prevalence in *L. fulica* from the area. This study aims to determine the prevalence of helminths in *L. fulica* collected from urban areas of Kota Samarahan, Sarawak.

**Methods:** The mucus and faeces of the snails were analysed for helminths using direct microscopy, sodium chloride floatation, centrifugal sedimentation and Harada–Mori filter paper culture.

**Results:** As a result, 66 (44%) out of 150 individuals tested were positive for helminth infections. Sixteen helminth species were identified, comprising 13 nematode species, two cestode species and one trematode species.

**Conclusion and importance:** The insights from this study will raise awareness of the negative impact of *L. fulica* on public health and the importance of controlling its population.

## KEYWORDS

helminth, Kota Samarahan, *Lissachatina fulica*, urban areas, zoonotic

## INTRODUCTION

Zoonotic diseases are infectious diseases of animal origin caused by bacteria, viruses, parasites or fungi that can be transmitted from animals to humans.<sup>1</sup> Among these, snail-borne parasitic diseases (SBPDs) remain a major public health concern globally, especially in low-income countries.<sup>2</sup> Snails serve as vectors and intermediate hosts for various zoonotic parasite species, such as *Paragonimus westermani*, *Fasciola hepatica* and *Clonorchis sinensis*, facilitating the transmission of these parasites to humans.<sup>2–4</sup>

*Lissachatina fulica*, commonly known as the giant African land snail, is an invasive terrestrial species native to East Africa.<sup>5</sup> It has established populations outside its native regions and has become alien or invasive. According to the global invasive species database, this species is the second-worst invasive alien species.<sup>6</sup> Gippet et al. highlighted its potential epidemic source because of multiple criteria: (i) vector of rat lungworm (*Angiostrongylus cantonensis*); (ii)

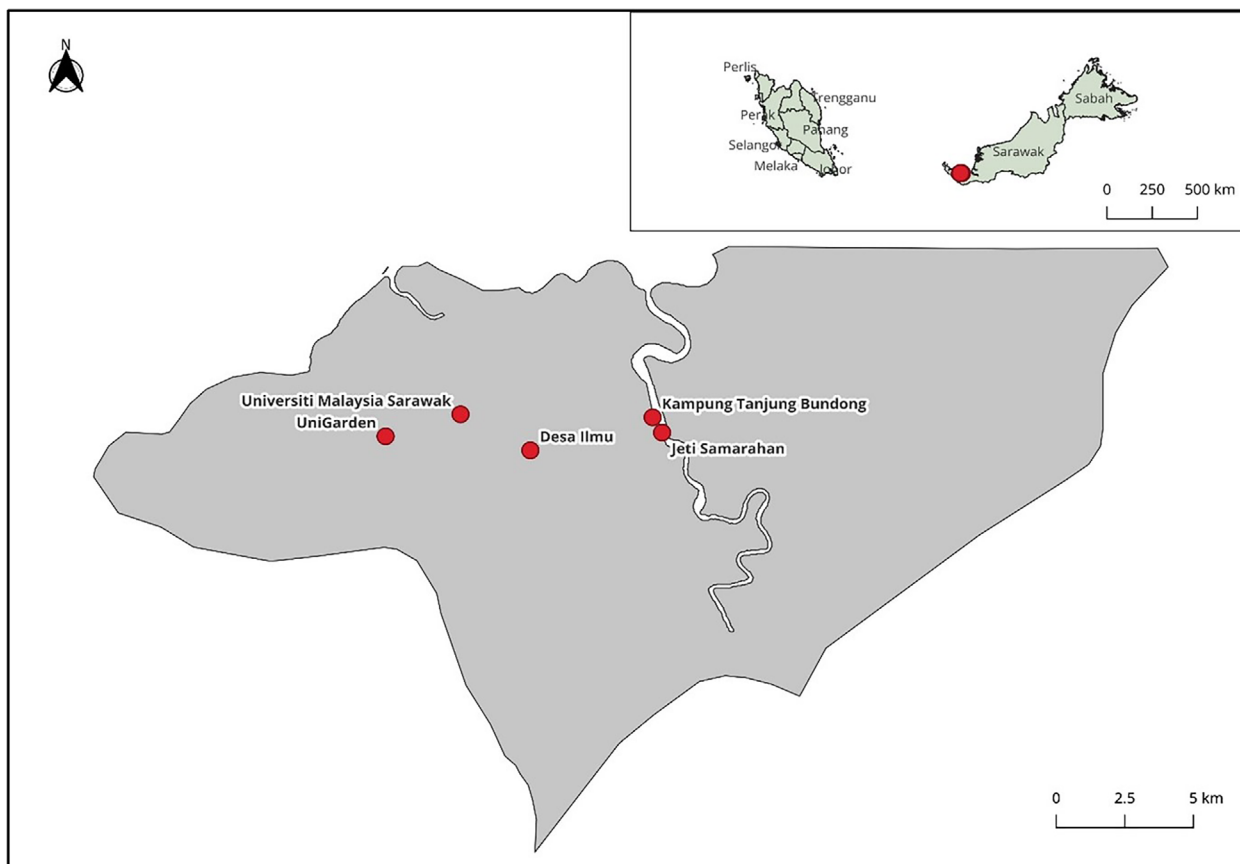
spreading globally beyond its native range in East Africa; and (iii) traded as an exotic pet.<sup>7–11</sup>

This species is invasive in Malaysia and able to thrive in urban environments because of its great reproductive capability and adaptation.<sup>12,13</sup> Kota Samarahan is a town in the state of Sarawak, East Malaysia (Borneo). The area has experienced rapid urbanisation and is recognised as the state's educational hub. The town's emergence as an educational hub presents an opportunity for the development of commercial and residential areas.<sup>14</sup> This urban growth increases opportunities for human–snail interaction, which may elevate the risk of pathogen transmission associated with *L. fulica*.

Despite these risks, the helminth prevalence of urban *L. fulica* populations in the area with relation to environmental factors has not been determined. Therefore, the main aim of this study was to determine the helminth prevalence from *L. fulica* in the urban areas of Kota Samarahan, Sarawak, and relate it to the environmental factors in the tropical region.

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**FIGURE 1** Smaller map shows Malaysia and Kota Samarahan, Sarawak (red dot). The larger map shows specific sampling sites (red dots) in urban areas of Kota Samarahan, where *Lissachatina fulica* was collected. Map was created using QGIS software (version 3.42).

**TABLE 1** Coordinates for each sampling locality in urban areas of Kota Samarahan, Sarawak.

Locality	Latitude (N)	Longitude (E)
Universiti Malaysia Sarawak	1°28'0.22"	110°25'30.51"
UniGarden	1°28'2.37"	110°25'1.06"
Desa Ilmu	1°26'49.44"	110°27'32.80"
Jeti Samarahan	1°27'34.98"	110°30'5.16"
Kampung Tanjung Bundong	1°27'46.27"	110°29'56.86"

Abbreviations: DI, Desa Ilmu; JS, Jeti Samarahan; KTB, Kampung Tanjung Bundong; UG, UniGarden; UNIMAS, Universiti Malaysia Sarawak.

## METHODOLOGY

### Study area

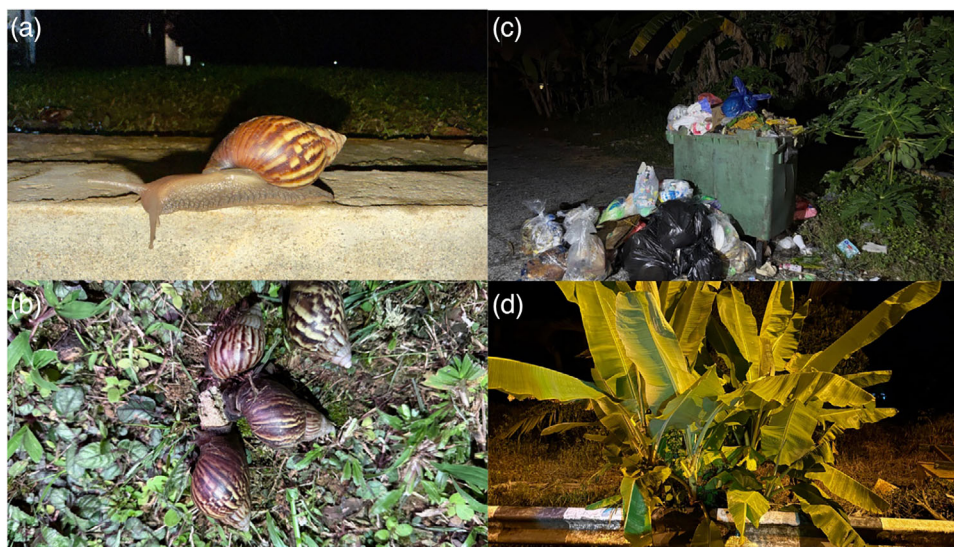
This study was conducted in the urban areas of Kota Samarahan, Sarawak. This area is characterised by flat, low-lying topography surrounded by peat swamps.<sup>14,15</sup> Daytime temperatures ranged from 28°C to 34°C, and the mean annual precipitation over 5 years was 4099.6 mm with a mean relative humidity of 85.2%.<sup>16,17</sup> The sampling sites were chosen based on the presence of residential or commercial areas, gardens or public areas where the snails were commonly found. A map of sampling localities was created using QGIS software version 3.42 (Figure 1). Table 1 shows the coordinates for each locality.

### Sample collection

Thirty individuals were collected from each of the five localities ( $n = 150$ ) between January and May 2023 during the northeast monsoon and inter-monsoon phases. This period has higher precipitation in western Sarawak, making it ideal for snail sampling due to high humidity.<sup>18</sup> Snails were hand-picked at night from 7:00 p.m. to 10:00 p.m. from gardens, crevices, waste areas and other potential shelter sites, as shown in Figure 2. Each snail was kept in a zip-lock bag, labelled with a field code, collection date and location. Proper handling techniques, such as using gloves and sanitation, were applied to prevent contamination between samples and handlers. Environmental parameters, including temperature and humidity, were recorded using an Extech environmental meter (model 45170), as shown in Table 2.

### Specimen handling, identification and measurement

The samples were transported at ambient temperature to the Parasitology Lab, Faculty of Resources and Science Technology, Universiti Malaysia Sarawak, the following morning for identification, measurement, and parasitological analysis. Each individual was identified morphologically as *L. fulica* using an identification tool<sup>19</sup> and weighed using a digital scale. The average weight of snails collected was  $24.81 \pm 6.81$  g (range: 10.90–44.00 g). Detailed data of snails' weight



**FIGURE 2** *Lissachatina fulica* and its collection habitats in Kota Samarahan, Sarawak. (a) Adult *L. fulica* specimen. (b) Cluster of snails in a garden habitat. (c) Snail collection site near the waste disposal area. (d) Snail collection site near banana plants.

**TABLE 2** Summary of environmental conditions (temperature, humidity and precipitation) recorded at each study site during snail collection in Kota Samarahan, Malaysia.

Site	Temperature (°C)	Humidity (%)	Precipitation type
UNIMAS	24.7–25.0	94.19–96.19	Rain
UG	29.0–29.8	71.50–79.06	None
DI	22.8–23.1	88.21–90.11	Rain
JS	27.0–28.0	83.79–88.84	None
KTB	25.0–26.0	88.76–94.19	Rain

Abbreviations: DI, Desa Ilmu; JS, Jeti Samarahan; KTB, Kampung Tanjung Bundong; UG, UniGarden; UNIMAS, Universiti Malaysia Sarawak.

are provided in [Supporting Information](#). All the data were recorded and analysed using Microsoft Excel and RStudio.

## Parasitological analysis and identification

Mucus and faecal samples from individual *L. fulica* were collected for analysis. Mucus was collected using a sterile glass rod and diluted with double-distilled water (ddH<sub>2</sub>O). Fresh faecal samples were obtained non-invasively by collecting excreted faecal matter and transferring it into individual sterile centrifuge tubes for further analysis.

The collected mucus was examined using the direct wet mount method, using methylene blue as a stain. The faecal examination was performed using three methods: sodium chloride (NaCl) flotation, centrifuge sedimentation and Harada–Mori filter paper culture. Briefly, for NaCl flotation, approximately 2 g of faecal material from each individual was placed in an individual tube and filled with NaCl solution (Merck KGaA). A coverslip was then placed on top of it. The samples were left for 15 min before microscopic examination. Centrifugal sedimentation involved centrifuging the faecal sample at 1500 rpm for 5 min before examining the sediment under a microscope. For the Harada–Mori filter paper culture, approximately 5 g of faeces was smeared onto a strip of filter paper. The strip was then placed in a tube filled with ster-

ile ddH<sub>2</sub>O and held upright for 7 days at room temperature. During incubation, motile larvae migrated to the water, which was examined after 7 days. Figure 3 provides the workflow of examination.

All methods were performed following standard parasitological protocols.<sup>20,21</sup> All wet mounts were examined immediately under a compound light microscope (Leica DM 500) at 40× and 100× magnification. All positive samples were identified to the lowest taxonomic level using published identification keys and reference books, such as Colour Atlas on Medical Parasitology<sup>22</sup> and Keys to the Nematode Parasites of Vertebrates: [Supplementary Volume](#).<sup>23</sup>

## Statistical analysis

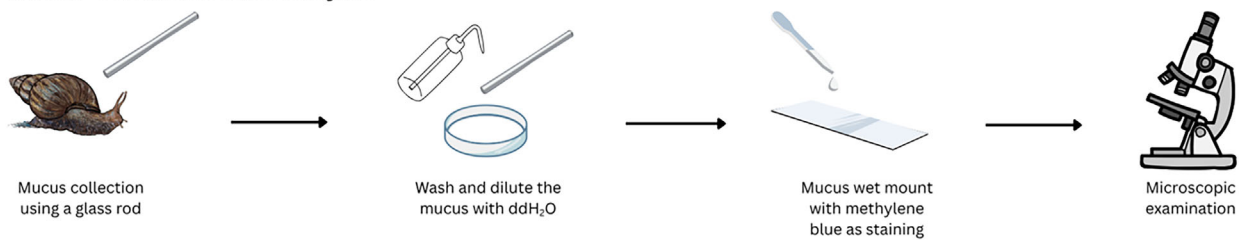
All the statistical analyses were performed using R software version 2025.09.0+387. Helminth parasite prevalence of *L. fulica* was calculated using the following formula: (positive snails/total snails) × 100.<sup>24</sup> Site-level prevalence and corresponding 95% confidence interval (CI) were calculated using Wilson's method due to moderate sample size ( $n = 30$  per site), while the Wald's method was used for the overall prevalence CI. A chi-square test was used to determine differences in prevalence among sites. A generalised linear model was applied to identify biological and environmental factors influencing infection likelihood. Significant level was determined at a  $p$ -value of less than 0.05.

## RESULTS

### Prevalence and spatial analysis

The overall prevalence of helminths was 44% (CI: 35.9–52.3). Sixteen helminth species were identified, belonging to two phyla (Nematoda and Platyhelminthes). Of these 16 species, eight were considered zoonotic species, as shown in Table 2, including *Strongyloides* spp., hookworm, *Oesophagostomum* spp., *Toxocara* sp., *Ascaris* sp., *Diphyllobothrium* sp.,

### Mucus Collection and Analysis



### Faecal Collection and Analysis

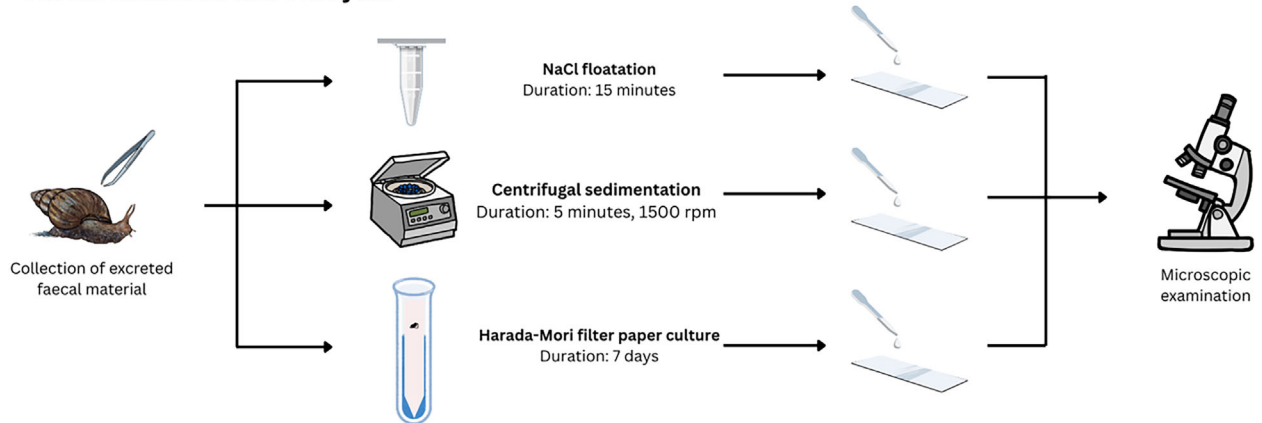


FIGURE 3 Workflow of parasitological examination of *Lissachatina fulica* in Kota Samarahan, Sarawak.

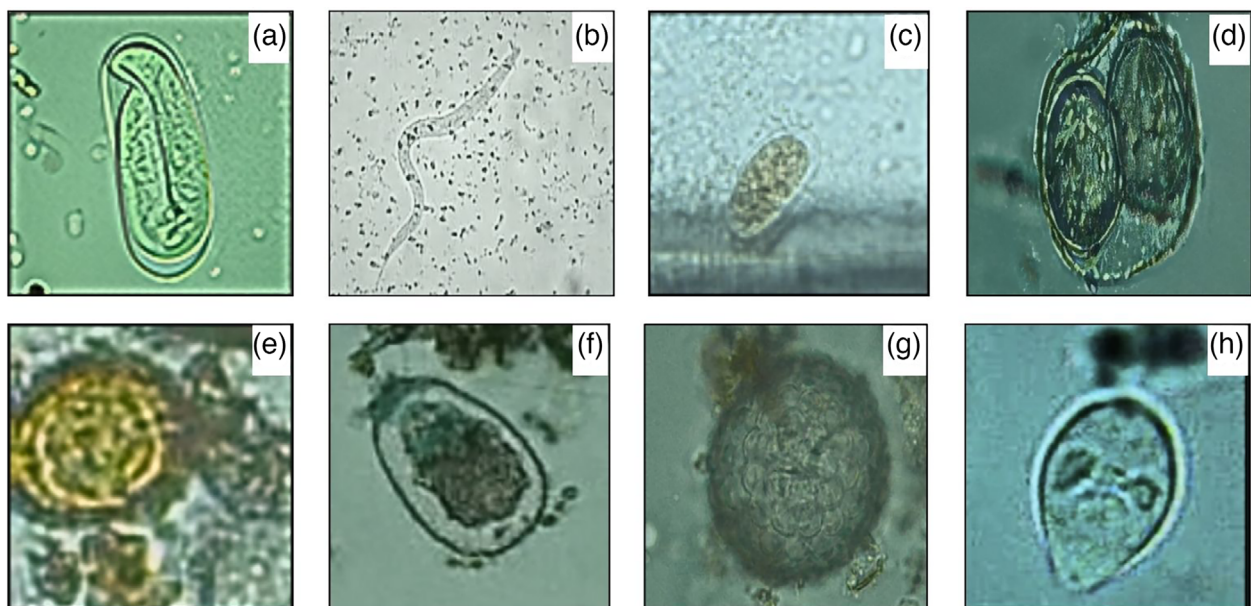


FIGURE 4 Zoonotic helminth of *Lissachatina fulica* in Kota Samarahan, Sarawak. (a) *Strongyloides* spp. (100 $\times$ ), (b) hookworm (40 $\times$ ), (c) *Oesophagostomum* spp. (100 $\times$ ), (d) *Toxocara* sp. (100 $\times$ ), (e) *Ascaris* sp. (100 $\times$ ), (f) *Diphyllbothrium* sp. (100 $\times$ ), (g) *Dipylidium* sp. (100 $\times$ ) and (h) *Dicrocoelium* sp. (100 $\times$ ). Images were captured using a compound light microscope at 40 $\times$  and 100 $\times$  magnification.

*Dipylidium* sp. and *Dicrocoelium* sp. (Figure 4). The most prevalent helminths were *Strongyloides* spp. (16%), hookworm (10%), *Rhabditis* spp. (4%) and *Oesophagostomum* spp. (2.7%).

Other than that, the prevalence of helminths varied among sampling locations. The location with highest prevalence was Jeti Samarahan (63.3%; 95% CI: 46.1–80.6), followed by Kampung Tanjung Bundong (53.3%; 95% CI: 35.5–71.2), Universiti Malaysia Sarawak (40.0%; 95% CI: 22.5–57.5), Desa Ilmu (33.3%; 95% CI: 16.5–50.2) and UniGarden (30.0%; 95% CI:

13.6–46.4). A chi-square test revealed that helminth prevalence was significantly different among sites ( $\chi^2 = 9.5779$ ,  $df = 4$ ,  $p$ -value = 0.04817).

### Environmental correlation analysis

Generalised linear model was used to determine the influence of environmental variables on helminth infection. The model showed that the mean humidity was the only significant

TABLE 3 Helminth identified from *Lissachatina fulica* in urban areas of Kota Samarahan, Sarawak.

Class	Species	Infected (n)	Prevalence (%)
<b>Phylum nematoda</b>			
Chromadorea	<i>Strongyloides</i> spp. <sup>a</sup>	24	16.0
	Hookworm <sup>a</sup>	15	10.0
	<i>Rhabditis</i> spp.	6	4.0
	<i>Oesophagostomum</i> spp. <sup>a</sup>	4	2.7
	<i>Aelurostrongylus</i> spp.	3	2.0
	<i>Cooperia</i> spp.	2	1.3
	<i>Quinisulcius</i> spp.	2	1.3
	<i>Dictyocaulus</i> spp.	2	1.3
	<i>Toxocara</i> sp. <sup>a</sup>	1	0.7
	<i>Strongylus</i> sp.	1	0.7
	<i>Ascaris</i> sp. <sup>a</sup>	1	0.7
	<i>Protostrongylus</i> sp.	1	0.7
	<i>Diaphanocephalus</i> sp.	1	0.7
<b>Phylum plathelminths</b>			
Cestoda	<i>Diphyllobothrium</i> sp. <sup>a</sup>	1	0.7
	<i>Dipylidium</i> sp. <sup>a</sup>	1	0.7
Trematoda	<i>Dicrocoelium</i> sp. <sup>a</sup>	1	0.7
		66	44.0

<sup>a</sup>Helminth of public health importance.

environmental factor influencing infection ( $\beta = 0.112$ , SE = 0.046,  $z = 2.42$ ,  $p = 0.015$ ); meanwhile, snails' weight, temperature and rainfall presence were not significantly influencing infection likelihood ( $p > 0.05$ ).

## DISCUSSION

*Lissachatina fulica* population in urban areas of Kota Samarahan exhibited a high helminth prevalence, with an overall infection of 44% and a predominance of nematodes. The detection of eight zoonotic species such as *Strongyloides* spp., hookworms and *Oesophagostomum* spp., indicates that *L. fulica* is frequently exposed to soil- and faecal-associated parasites. To the authors' knowledge, this is the first study to examine multiple helminth species of *L. fulica* in Malaysian Borneo.

The predominance of *Strongyloides* spp. in this study aligns with previous studies from other tropical regions,<sup>25–28</sup> which can be attributed to the soil-dwelling nature of both *Strongyloides* spp. and *L. fulica*. The detection of hookworm in this study is consistent with previous reports from Venezuela and Brazil,<sup>29,30</sup> whereas the presence of *Oesophagostomum* spp. contradicts a report.<sup>27</sup> In that report, Liboria et al. detected *Oesophagostomum* spp. from *Archachatina marginata* but absent in *L. fulica*. The differences between the previous report and this study may result from variations in environmental conditions and methodological approaches rather than a true absence. Additionally, *L. fulica* also shows a habit of feeding on faeces, which may explain the acquisition of both parasites.<sup>31</sup> Orico et al. reported that infection of *L. fulica* with *Ancylostoma caninum*, a type of hookworm, is rare. The detection of the parasites resulted from the contact between the snails and contaminated animal faeces.<sup>29</sup>

*Angiostrongylus cantonensis* remained undetected despite a previous report of eosinophilic meningitis from Sarawak, suspected to originate from *L. fulica*.<sup>32,33</sup> This inconsistency is likely to stem from a limited sample size or study area coverage. Methodological limitations may also affect detection outcomes. Future research should increase the sample size, expand study areas, and use a more sensitive method, such as the polymerase chain reaction, targeting the region of the ribosomal DNA spanning the 18S 3' end, the internal transcribed spacers (ITS1 and ITS2), 5.8S and 28S 5' end, using nematode universal primers N93 (forward primer: 5'-TTG AAC CGG GTA AAA GTC G-3') and N94 (reverse primer: 5'-TTA GTT TCT TTT CCT CCG CT-3') for accurate detection of *A. cantonensis*.<sup>34,35</sup> These approaches will provide a more comprehensive understanding of its distribution and prevalence.

Environmental modelling identified humidity as a significant environmental factor in this study. Increased humidity and temperature can enhance parasite survival and development in the environment and host organisms.<sup>36</sup> Elevated temperature and persistent humidity in the urban areas of Kota Samarahan provide favourable conditions for the parasites to persist in both environment and hosts, which may have contributed to the high prevalence recorded in this study.

Eight helminth species identified in this study are zoonotic (refer to Table 3), capable of causing various infections to humans and animals. Urbanisation provides a suitable environment for *L. fulica* to survive and proliferate in urban areas,<sup>37,38</sup> increasing the likelihood of exposure among residents to the pathogens carried by the snails. Children are especially at higher risk of contracting an infection, as they are more likely to lick the snails or their contaminated hands.<sup>39</sup> These findings underscore the public health risk related to *L. fulica* in Kota Samarahan and emphasise the importance of

*L. fulica* as a sentinel species to monitor environmental contamination by virulent pathogens. Infection levels can indicate parasitic contamination in urban areas, aiding public health assessment and disease surveillance.

Several limitations must be noted regarding this study. First, this study used a relatively smaller sample size compared to other similar studies. Second, helminth identification relies solely on morphological characteristics, although widely used, can lead to misidentification and may underestimate helminth species diversity and abundance compared to molecular methods. Third, this study was conducted only during the Northeast Monsoon, so no potential seasonal variations between the dry and wet seasons can be observed, unlike other studies. This study only examined the mucus and faeces of *L. fulica* without tissue dissection or digestion, as whole organisms were used in heavy metal studies, likely underestimating helminth diversity and abundance.

## CONCLUSION

This study demonstrated that the urban populations of *L. fulica* in Kota Samarahan, Sarawak, harbour diverse helminth species. Several zoonotic species were recorded in *L. fulica*, comprising mostly of nematodes. These findings contribute valuable insights on SBPDs in Malaysian Borneo. This study has implications for public health, indicating potential exposure risk to the residents, especially children, and urges the need for monitoring and control strategies. Future research should implement a larger sample size, expand study areas, integrate morphological and molecular identification, incorporate tissue dissection or artificial digestion, and study seasonal effects to better understand helminth diversity and transmission dynamics. Additionally, it is also recommended to expand similar studies to other gastropod species to identify other potential helminth carriers. The insights gained from this study may be of assistance in developing monitoring and control strategies, as well as improving awareness among the public regarding the medical and economic impacts of *L. fulica*.

## AUTHOR CONTRIBUTIONS

Farid Farhan Mohd Nor, Nur Aniyah Sulaiman and Tiffany Junior Singgah Iddie collected the samples and performed data curation. Farid Farhan Mohd Nor and Madinah Adrus performed identification and statistical analysis. Farid Farhan Mohd Nor wrote and drafted original manuscript. Madinah Adrus and Dayang Norafizan Awang Chee designed the project review and editing. Madinah Adrus validated the data of identification and performed project administration, supervision, visualisation, reviewing and editing of the manuscript.

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## CONFLICTS OF INTEREST

The authors declare they have no conflicts of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study can be acquired from the corresponding author upon reasonable request.

## ETHICS STATEMENT

This study does not require any animal ethics since it used invertebrates as a model of study.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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