Screening *Strongyloides spp.* Infection from Wild Rodents Implications for Public Awareness and Attitudes on Zoonotic Diseases in Malang City, Indonesia

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Abstract

Strongyloidiasis is a nematode parasite with broader distribution proved to infect humans and animals. *Strongyloides ratti* common endoparasites infected rodent as the most adaptive population in various environments. We have currently raised concerning the neglected tropical diseases (NTDs) situations in Indonesia. This study aimed to understand the incidence of *Strongyloides spp.* infecting wild rodents in Malang City during October to December 2021 by stratified random sampling. A total of 50 rats (*Rattus norvegicus*, *Rattus rattus diardii*, *Mus musculus)*, 26 male (52%) and 24 female (48%). Following 12 juveniles (24%) and 38 adults (76%). Coprology examinations use floatation and sedimentation methods immediately after the gastrointestinal tract's stool collection (GITs). We also administered a limited survey to get responses from 80 people (housewives, sellers, employee, and students) to obtain risk transmission, public awareness, and attitudes. We were continually analyzed data using the chi-squared and Fisher Exact Test. The microscopic examination of stools was 28% positively detected *S. ratti.* Our study found an association presented between the age of rats and Strongyloidiasis infections ($p<0.05$). However, the gender of rats did not have a significant association ($p>0.05$*)* to the *S. ratti* several infections. In addition, most participants did not understand the zoonotic disease and these infections, indicating a low-level knowledge 71 (88%), because better education supports the increase of awareness. However, almost all participants have good practice of hygiene and sanitation toward COVID-19 situations 72 (90%). The further study recommends investigating Strongyloidiasis infections in another species and increasing the education program for housewives and sellers in a traditional marketplace to have better knowledge.

Keywords: Malang, rodent, strongyloidiasis

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INTRODUCTION

The epidemiological effect of neglected tropical diseases (NTDs) transition, especially in Indonesia elevated 111 million people considered the effect on helminth infection. This disease impacts not only the health sector but also the economic sector in this country (Tan *et al*., 2014). Currently, Indonesia's remaining challenges to the health system include environmental threats, a rapidly growing population, significant pollution, clean water and sanitation, housing condition, and animal-borne diseases from the village level to an urban area that adverse situations (WHO, 2021). In global prevalence, strongyloidiasis is a natural neglected tropical disease (NTDs) that affects most people in poor rural communities in the tropical and sub-tropical regions. The prevalence of global infection in 2017 was over 8,1% in endemic areas (Wardell *et al*., 2017; Buonfrate *et al*., 2020). The prevalence of strongyloidiasis infection has not yet been reported in Indonesia (Nugrahani *et al*., 2017).

The rat in the environment still exists and acts as a reservoir of pathogens especially endoparasites (Kusuma *et al*., 2021). Moreover, the problem is the ability of rodent that rapidly

spread zoonoses disease and have been roled in significant epidemics (Jittapalapong *et al*., 2009; Mawanda *et al*., 2020). The numerous populations of wild rats wherever densely populated, like cities, suburban, urban areas, and agricultural areas, result from a commensal association between rats and humans to obtain food, materials of human animal origin, water, and habitats (Kataranovski *et al*., 2011). The common endoparasites of rodents came from genus *Strongylus* are *S. ratti and S. venezuelensis* (Breloer and Abraham, 2017).

These gastrointestinal endoparasites have a complicated life cycle because it has two adult generations – one in the host and the free-living stage (Infective L3s). In general, adult stages is female-only and reproduction by parthenogenesis (invertebrate animals) (Viney, 2017). The previous study considered *Strongyloides ratti* (Sr) an ideal experimental infection model for Strongyloides research and understanding their life cycle. However, there are no known natural infections of wild rats based on age and sex (Viney, 2017; Viney & Kikuchi, 2017). The most-reported natural infection of *S. ratti* (Sr) from brown rats (*Rattus norvegicus*) in the Grenada, West Indies (43.2%), than infective larvae mostly predilection in large intestine Japan, and Malaysia (Al-Zihiry *et al*., 2015; Coomansingh-Springer *et al*., 2019; Shintoku *et al*., 2005). Commonly, their transmission is associated with demographic factors, sanitation conditions, and personal hygiene. In Indonesia limited reports about this infection on other species of wild rats yet (Tangpong *et al*., 2021).

Malang is the second most populated city that is a potential urban area in East Java. Compared with other cities, people who live in Malang have multiple jobs and careers (Soseco, 2011). Before planning an education program for strongyloidiasis infection, there are essential to consider the factor of people in Malang city to obtain risk transmission, public awareness, and attitudes toward zoonotic disease, which has more human population compared to other in Indonesia by limited survey (Tangpong *et al*., 2021). This study aimed to investigate the incidence of *Strongyloides spp. i*nfecting wild rodents and to assess public knowledge, awareness, and attitude on zoonotic diseases and Strongyloidiasis in urban areas, Malang City, Indonesia.

MATERIAL AND METHOD

Ethical Statement

This study was approved by the Institutional Animal Care and Use Committee of the Brawijaya University, Malang (Registry. No: 090-KEP-UB-2021). All protocols and procedures for trapping and handling of animals in this study are accordance with the direction of the Ethics Committee, BrawijayaUniversity.

Trapping Locations, Sampling

The study was conducted in Malang City, Indonesia. A total of 50 rats (*R. norvegicus, Rattus rattus diardii*, *Mus musculus)* consist of 26 males (52%) and 24 females (48%) for pilot study. The wild rats were collected randomly from the different urban areas determined by both food resource availability and near a public space with the rats population lived (e.g., market, restaurant, and residence) from October to December 2021. All rodents were captured live using a rectangular trap baited with peanuts or roasted coconut. Animal capture is based on the guideline of the American Veterinary Medical Association (AVMA, 2020).

Limited Survey Instrument

The questionnaire was designed in this pilot study to get valid responses to understand the meaningfulness of the concept of public health from 80 people with various career respondents, such as housewives, sellers, employees, and students. The demographic questionnaire included information about gender, age, education, and profession. The educational background of this study was determined by stratified random sampling. The respondents were asked to obtain data about knowledge of zoonosis disease consisting of risk transmission, public awareness of zoonosis, and attitudes. The structured questionnaire consisted of 20 items to evaluate knowledge (8), attitude (6), and (6) public awareness related to zoonotic disease risks

from Strongyloidiasis. The written consent was taken from the respondents to publish their data (Islam and Ahmed, 2019).

A total score transforms to 100-80 for "good knowledge and high awareness," 50-70 for "moderate," and less than 50 for "poor knowledge and low awareness." Responses for knowledge items were measured into "correct" and "incorrect." Public awareness was measured into "undesirable" by combining "strongly disagree" and "disagree" reactions and a "desirable" attitude by combining "strongly agree" and "agree" responses. "Neither disagree nor agree" replies were removed during the analysis. Attitude response evaluation by their activity reflects good behavior "yes-success" and bad behavior "no failure." (Table 1) (Kusumarini *et al*., 2020; Alemayehu *et al*., 202).

Animal Identification

Rodents species were collected by box and transported to the Laboratory of Veterinary Parasitology Universitas Brawijaya. All rats were euthanized using ketamine 75 mg/kg body weight and xylazine 10 mg/kg body weight. This anesthetized drug is administrated through intraperitoneal injections. Moreover, they identified based on morphological measurements. That physical performed inspection with external unit head length (H), total body length (TL), length of the tail (T), hindfoot length (HF), ear length (E) in millimeters and weight in grams, and maturity determination of development stage consist of juvenile, sub-adult, and adult. It can help to correctly identify each animal to the species level based on characteristics (Herbreteau *et al*., 2011). Female rodents have mammae organs in three locations (pectoral, post axillary, and inguinal). The formula pairs of nipple *R. norvegicus*, *R. rattus diardii*, and *M. musculus* were directly shown below (1+2+3),(1+1+3), and (1+2+2), respectively. It is crucial to identify each species of wild rodent that capture correctly (Herbreteau *et al*., 2011; Yuliadi *et al*., 2016).

Identification of *Strongyloides spp.*

After sacrificing the wild rats following dissecting the abdominal cavity, We carefully removed the gastrointestinal tract and opened it lengthwise to collect a fecal sample. We continued to inspect the duodenum to separate helminth with feces. Sample directly Coprology examinations use fecal floatation in a saturated sugar solution, and sedimentation methods immediately after the gastrointestinal tract's stool collection (GITs) seem to be the most sensitive test (Beugnet *et al*., 2018).

The flotations were screened for the appearance of eggs using a microscope Olympus CX-21 (Olympus Corp., Tokyo, Japan) with 100x-400x magnification following captured used Opti Lab Advanced Plus camera (Miconos Corp., Yogyakarta, Indonesia). The eggs morphometric evaluation difference of two categories emphasis of granulated embryos such as Granulated embryo eggs and larval eggs. Larval stages consists of three stadium (The L_1 rhabditiform, L_2 rhabditiform, L_3 filariform). After identifying eggs and larval stages of *Strongyloides spp.,* the positive sample was measured by ImageJ software to know the average length of larva and eggs (Taylor, Coop, and Wall 2007; Tobar *et al*., 2021).

Statistical Analysis

All collected data was conducted using the Chi-squared and Fisher Exact Test (FET) with a $P\text{-}value < 0.05$ regarded as statistically significant. Analyses were performed with SPSS®21 for Windows. To determine public knowledge and attitude in Malang city, Indonesia, the data from the limited survey were analyzed using descriptive in this study (Alho *et al*., 2018).

RESULT AND DISCUSSION

Study Population Characterization

A total of 50 rodents were captured from eight locations in Malang city (Figure 1). The single-life trap is located in various locations involve of three traditional and semi-modern markets, five places near the human residence, and an urban area. This study focuses on four sub-districts in Malang city i.e. Klojen, Sukun, Blimbing, and Wagir.

Table 1. Knowledge, public awareness, and attitudes toward zoonotic diseases item description

No	Item content	Response
	Knowledge subscale items	
K1	Infectious diseases can be transmitted from animals to humans or humans to animals.	Correct/Incorrect
K2	Identified the name of three diseases correctly that include neglected tropical diseases.	Name/Not name
K3	Identified the name of human pathogenic nematode that infected worldwide.	
K4	Strongyloidiasis can infect humans and animals.	Correct/Incorrect
K5	You can get infection from environment contaminated feces from rodents sick.	Correct/Incorrect
K6	Food and water that contamination with feces rodents can transmitted infectious disease.	Correct/Incorrect
K7	Rodents can act as reservoir of Strongyloidiasis.	Correct/Incorrect
K8	Human infection is primarily acquired by the filariform larvae.	Correct/Incorrect
	Public awareness subscale items	
PA1	Contact with soil potentially transmits free-living larvae from soil to you.	SDA/DA/N/A/SA
PA2	Children can transmit strongyloidiasis if walking barefooted.	SDA/DA/N/A/SA
PA3	The spread of infective larva in the environment caused by wild rodents.	SDA/DA/N/A/SA
PA4	Zoonotic infection almost spread in animals.	SDA/DA/N/A/SA
PA5	Strongyloidiasis is preventable if you can manage hygiene and sanitation.	SDA/DA/N/A/SA
PA6	Strongyloidiasis is a severe disease and need serious attention.	SDA/DA/N/A/SA
	Attitude subscale items	Ys/Nf
A1	Avoid any contact with rodents materials.	Ys/Nf
A2	Always cover your foots while walking on soil.	Ys/Nf
A3	Dispose of material that contacts rodent's feces.	Ys/Nf
A4	Wash hands with soap and water flow after contact with rodents or soil.	Ys/Nf
A5	Wash any vegetables and fruits before consumption.	Ys/Nf
A6	Avoid putting your hands in your mouth after contact or playing on ground.	Ys/Nf

SDA = Strongly Disagree; DA = Disagree; N = Neutral; A = Agree; SA = Srongly Agree
Ys = Yes-success; Nf = No-failure.

Table 2. Proportion of *Strongyloides spp.* infection in rats at Malang city, Indonesia

Rat species n=50	No.+ve (%)	No.-ve (%)	T.S (%)
R. rattus diardii	8 (16%)	23(46%)	31 (62%)
R. norvegicus	6 (12%)	9 (18%)	15 (30%)
M. musculus	0	3 (6%)	3 (6%)
Suncus murinus	0	1(2%)	1 (2%)
O.P	14 (28%)	36 (72%)	50 (100%)

Note: No.+ ve: number positive; No.- ve: number negative; O.P: overall proportion; T.S: total sampel; P%: Prevalence.

Table 3. Proportion of *Strongyloides spp.* positive infection in rats according to species and age

Rat species	No.+ve (%) Juvenile	No.+ve (%) Adult	T.P (%)
R. rattus diardii	3(6%)	5(10%)	8 (16%)
R. norvegicus	5 (10%)	1 (2%)	6 (12%)
M. musculus	0	0	0
Suncus murinus	0	0	0
O.P	8 (16%)	6(12%)	14 (28%)

Note: No.+ ve: number positive; O.P: overall proportion; T.P: total proportion; P%: Prevalence.

Table 4. Proportion of *Strongyloides spp.* positive infection in rats according to species and sex

Rat species	No.+ve (%) male	No.+ve (%) female	T.P (%)
R. rattus diardii	2 (4%)	6 (12%)	8 (16%)
R. norvegicus	3 (6%)	3 (6%)	6 (12%)
M. musculus	0	0	0
Suncus murinus	0	0	0
O.P	5 (10%)	9 (18%)	14 (28%)

Note: No.+ ve: number positive; O.P: overall proportion; T.P: total proportion; P%: Prevalence.

Table 5. Socio-demographic characteristics and distributions of educational background of the respondents at Malang city, Indonesia

Variabel		Number	(%)
Sex	Male	32	40.00
	Female	48	60.00
Age	Less than 15	5	6.25
	Between 15-30	12	15.00
	Between 31-50	34	42.50
	Greater than 50	29	36.25
Profession	Employee	4	5.00
	Housewive	24	30,00
	Seller	47	58.75
	Student	5	6.25
Educational background	Illiterate	5	5.25
	Primary school	27	33.75
	Secondary school	25	31.25
	High school	17	21.25
	Colleges and University	6	7.50

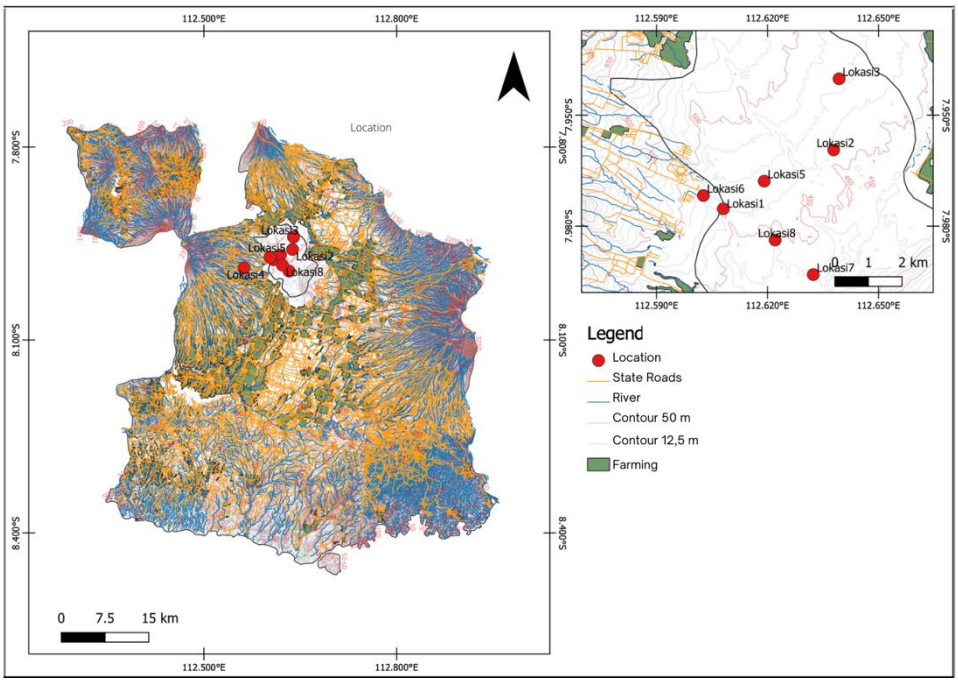

Figure 1. The study area in Malang City determined residential areas, marketplace, and public area suitability circled in red.

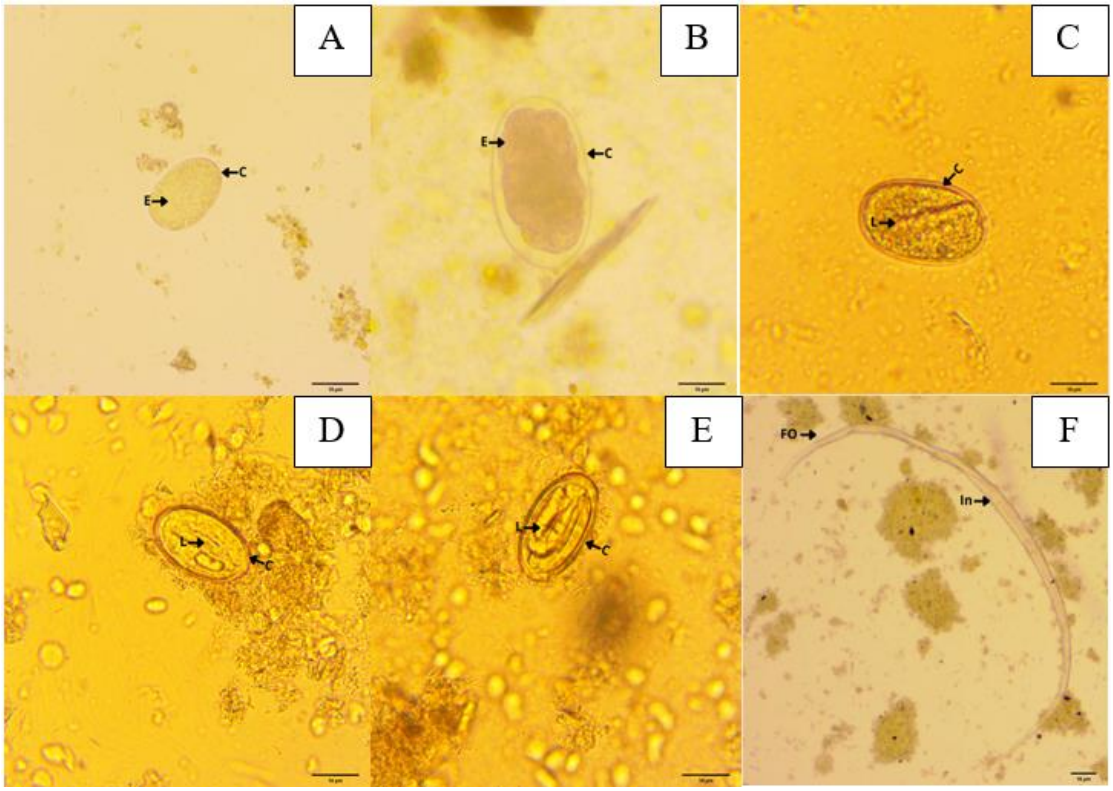

Figure 2. Development stages of *S. ratti* eggs obtained in feces of infected Black rats and Brown rats in Malang city identified by direct fecal smear method (100X-400X); A, B). Granulated embryo eggs. The eggs are thin-shelled, transparent and oval in shape. C, D, E). Larval eggs with lugol staining. The eggs contain active rhaditiform larvae that were ready to hatch. F). Third stages larva (iL_3) (scale bars: 10μm). E: Embryo; C: Cortex; L: Larva FO: Filariform esophagus; In: Intestine.

There are places at the center of economic growth with dense populations. Especially almost people have massive mobilization in this area. The wild rats sample collected from study sites including *R. rattus diardii* 31(62%), *R. norvegicus* 15 (30%), *M. musculus* 3 (6%) *and Suncus murinus* 1 (2%) (Table 2). Wild rodents have great diversity and similarities that make them challenging to identify. Furthermore, external morphology identification (head length, total body length, tail, hindfoot, and ear) is necessary for correct identification (Herbreteau *et al*., 2011).

A previous study showed slightly more *R. norvegicus* (37.5%) in the Blimbing sub-district, Malang city. Similarly, the literature widely reported that helminths are more often infected in Black rats (*R. rattus spp.*) than Brown rats (*R. norvegicus*) in urban area in Iran, Japan, Malaysia, and the Netherlands (Shintoku *et al.*, 2005; Pakdel *et al.*, 2013; Al-Zihiry *et al.*, 2015; Franssen *et al.*, 2016; Kusuma *et al.*, 2021). Black rats are common invation in urban area, the residential area and market places were favorable habitats which regularly contact with human. The disadvantage of this rodents were high reproductive capacity, easily transmitted Rodent-borne pathogens and propensity towards close association with humans (Battersby, 2002; Meerburg *et al*., 2009; Mustapha *et al*., 2019).

Identification of *Strongyloides spp.*

Based on 50 rat samples collected from study sites, 14 (28%) were found positive for *S. ratti* by microscopic examination (Figure 2). Parasitological analysis of stool samples collected from GITs revealed the presence of eggs with different stages of development. We could differentiate granulated embryo eggs in some stool samples, and the larval eggs contain active rhaditiform larvae moving inside. Moreover, we found third stages larva (iL3) nematode from all

samples. There were identified by characteristic features The eggs *S. ratti* had an oval shape with symmetric polar points and presented a thin-shelled chitinous cortex with a smooth surface. Sometimes the shelled looks transparent.

Additionally, they (n=14) had a width and length of 35.31µm x 52.44 µm for granulated embryo eggs and 59.19 µm x 31.06 µm for the larval eggs. A previous study reported that the eggs of hookworm species have a dimension of width was about half that of length. Other studies observed *S. venezuelensis* eggs that infected wild rodents have an average length of 43.22 µm and a width of 28.8 µm. These may have indicated that the width of hookworm eggs has twice of length. There is little information on different sizes of *S. ratti* in granulated embryo eggs since most reports of experimental approach Strongyloidiasis infections (Al-Zihiry *et al*., 2015; Tobar *et al*., 2021).

Our study found that the relation between age and infection was statistically significant ($p<0.05$). Based on the host age was slightly higher in juvenile 8 (16%) than adult 6 (12%) rats (Table 3). The results show that juvenile rats are more active in looking for food sources. This research follows the previous study in Malaysia (Tijjani *et al*., 2020). On the other hand, in urban ecosystems like Malang city, adult and juvenile rats have been potentially infected with endoparasites because of their social behavior. Moreover, young rats explore more actively, especially at night, whereas adult rats spill less food caused of their neophobia is more sensitive. Transmission of *S. ratti* infection often by iL3s penetrating host skin or trans-mammary transmission (Viney and Kikuchi, 2017; Clapperton, 2019; Kusuma *et al*., 2021).

The findings of this study show that the gender of wild rats did not have a significant association ($p>0.05$) with the infection (Table 4). The result is in line with that reported in Turkey that infection rate between male and female have no significant different (Gürler *et al*., 2011). Prevalence of *S. ratti* infection was not associated with the gender of wild rodents, which is in line with a study conducted by (Coomansingh-Springer *et al*., 2019) in the West Indies and (Franssen *et al*., 2016) in the Netherlands. But these findings contradict (Hancke *et al*., 2011) wheres found that males have a slightly higher endoparasites prevalence. Indonesia has a tropical climate with frequent rainfall, which potential of parasites to be transmitted. *S. ratti* and *S. venezuelensis* are common parasites, with *S. ratti* being ubiquitous, while *S. venezuelensis* is restricted to warmer climates (Viney, 2017). Another study in Southern Thailand and Laos reported that the prevalence of roundworm infections might be an environmental factor, including long rainy seasons, temperature, poor sanitation, and facilitated geographical characteristics (Anamnart *et al*., 2015; Vonghachack *et al*., 2015).

Assesment of Public Awareness, and Attitudes on Zoonoses

The results from the first survey indicate that most participants had 71 (88%) misconceptions about the zoonotic disease and these infections. However, almost all participants have good attitudes based on the practice of hygiene and sanitation toward COVID-19 situations worldwide 72 (90%). Our study found that housewive 24 (30%) and sellers 47 (58.75%) in the traditional market may confuse about various names of zoonoses disease and their transmission (Table 5). Additionally, most respondents have a poor educational background, especially in primary school 27 (33,75) and secondary school 25 (31,25%) whereas high school 17 (21,25%) and Colleges and universities 6 (7,50%). So, we assume their misperception of zoonotic disease relates to background education. Previous reports showed that better education increased the awareness and attitude of people. Furthermore, knowledge and personal hygiene practice are the main foundation for raising respondents' awareness about zoonosis diseases (Kusumarini *et al*., 2020).

Rodents perform potential mediators in the transmission pathogen. An urban area is a perfect place for possible hookworm transmission or other pathogens by wild rodents. The impact of this condition creates significant problems for human health and public health concerns, which

can cause accidental ingestion, larva infective exposed skin when contact with contaminated soil, eggs spreading in the environment, and contamination of food or agricultural product (Coomansingh-Springer *et al*., 2019; Islam *et al*., 2021; Kusuma *et al*., 2021).

In a nutshell, this study showed the significance to public health in Malang city, Indonesia, which can cause infection of zoonotic endoparasites in human populations, such as Strongyloidiasis. Therefore, it is necessary to consider the role of rodents in spreading infectious diseases such as environment, hygiene, and sanitation in Malang for their better control. Besides underlying all approaches to promoting public health for local people is a solid commitment to collective action and collaboration with the government to protect and promote the highest attainable standard of better health. Furthermore, increasing active participation and mentoring is necessary to attitude concern for zoonotic disease (Kusumarini *et al*., 2022; World Health Organisation, 2015).

CONCLUSION

This study showed that the incidence rate of *Strongyloides spp*. in Malang city, Indonesia, reached 28%. Therefore, extensive studies are required to investigate Strongyloidiasis infections and rodent-borne zoonotic pathogens in other species among the diverse human population and to increase the education program for housewives and sellers in a traditional marketplace to have better knowledge. The present study observed that the low educational background of professionals or non-health educated professionals is not conscious of zoonotic diseases. Further work should be taken to assess the prevention and control.

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