



# *R. rattus* and *R. norvegicus*, as reservoirs of zoonotic endoparasites in Ecuador

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Received: March 2020; Accepted: December 2020; Published: May 2021.

## ABSTRACT

**Objective.** The present study was to identify gastro-intestinal endoparasitic helminths in wild rats. **Materials and methods.** a parasitological study was carried out to know the helminth-fauna in different urban and rural areas in five provinces of the Republic of Ecuador, during 2014-2017. The rodents were captured and transported to the National Parasitology Reference Center of the National Institute of Public Health Research - Guayaquil, for further analysis. **Results.** 125/211 (59.2%) rats with endoparasites were found, 13/20 (65%) for *R. rattus* and 112/191 (58.6%) *R. norvegicus*. The most prevalent nematode: was *Nippostrongylus brasiliensis* for both species and *Heterakis spumosa*; followed by the cestodes: *Hymenolepis diminuta*, *H. nana*, *Moniliformis moniliformis* and *Cysticercus fasciolaris*. **Conclusions.** The presence of four species of zoonotic cestodes in the sampled rodents that live near human communities, represent a potential risk of infection for the inhabitants. Therefore, the control of the population of rodents in residential areas and the awareness of the local population about the risk of disease transmission through rodents seems to be totally necessary.

**Keywords:** Parasitosis; helminth; fauna; cestodes; nematode; zoonoses (Source: DeCS).

## RESUMEN

**Objetivo.** Recolectar información sobre la presencia de parasitosis intestinales en roedores, con énfasis en parásitos de importancia médica en humanos. **Materiales y métodos.** Se realizó un estudio parasitológico para detectar la presencia de helmintos en roedores en zonas urbanas y rurales de cinco provincias del Ecuador entre el 2014 y 2017. Los roedores fueron capturados y transportados al Centro Referencia Nacional de Parasitología del Instituto Nacional de Investigación en Salud Pública – Guayaquil, para su posterior análisis. **Resultados.** Se encontraron 125/211 (59.2%) ratas con endoparásitos, 13/20 (65%) *R. rattus* y 112/191 (58.6%) *R. norvegicus*. El nemátodo más prevalente fue *Nippostrongylus brasiliensis* (para ambas especies de roedores), y *Heterakis spumosa*, seguido por los cestodos: *Hymenolepis diminuta*, *H. nana*, *Moniliformis moniliformis* y

### How to cite (Vancouver).

Solórzano AL, Sánchez-Amador F, Sánchez GS, Pizarro VJ. *R. rattus* and *R. norvegicus*, as reservoirs of zoonotic endoparasites in Ecuador. Rev MVZ Córdoba. 2021; 26(3):e1260. <https://doi.org/10.21897/rmvz.1260>



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*Cysticercus fasciolaris*. **Conclusiones.** La presencia de cuatro especies de cestodos zoonóticos en los roedores muestreados que viven cerca comunidades humanas, representan un riesgo potencial de infección para los habitantes, especialmente en el caso de *R. norvegicus* (presento mayor diversidad de especies parasitarias). Por lo tanto, el control de la población de roedores en las áreas residenciales y las recomendaciones a la población local sobre el riesgo de transmisión de enfermedades a través de roedores parece ser totalmente necesario.

**Palabras clave:** Parasitosis; helminto; fauna; cestodos; nemátodo; zoonosis (*Fuente: DeCS*).

## INTRODUCTION

Economic development has led to increased urbanization, as people migrate from rural areas in search of employment and educational opportunities (1). In many developing countries, this, in turn, has led to increased urban poverty, which is reflected in poor housing, infrastructure support, and health and social services, characteristics that define slum settlements. (2). Although there have been initiatives to reduce poverty, such as the strategies undertaken to achieve the Millennium Development Goals, the number of residents in marginal neighborhoods increased by 28% from 1990 to 2014 (from 689 to 881 million) (1,2).

Rodents are recognized as hosts for at least 60 zoonotic diseases that pose a serious threat to humans (3,4,5). Historically, Asian rodents of the *Rattus* genus have been implicated in the emergence and spread of infectious diseases such as plague, murine typhus, typhus, leptospirosis, and hantavirus hemorrhagic fever, among others (6). They can cause considerable economic loss (7,8) and have a great impact on biodiversity (9,10).

Studies in Serbia reported 68.5% of the 302 rats with intestinal helminth infection. Seven helminth species were recovered, of which five were nematodes: *H. spumosa*, *N. brasiliensis*, *Capillaria* sp., *Trichuris muns* and *Syphacia muris*, and two species of cestodes: *H. diminuta* and *Rodentolepis fraterna*. The most prevalent parasites were *H. spumosa* (36.7%) and *H. diminuta* (30.5%) for *Rattus* spp. (11).

In Latin America, studies carried out in Medellín, Colombia, captured 254 specimens of *R. norvegicus* where *T. taeniaeformis cysticerci* were found in the livers of 6 specimens that presented larvae, with a frequency of 2.4% (6/254) (12).

Infection frequencies were reported by: *H. diminuta*, *Rodentolepis fraterna*, *Raillietina demerariensis*, *Aspicularis tetraptera*, *T. muris*, *Gongylonemaneo plasticum* and *M. moniliformis* in rats in urban areas of the city of Lima, Peru (13).

There is a lack of information on the impact they cause on the transmission of zoonotic etiologic agents in continental Ecuador; there are only records of the presence of *R. rattus* and *R. norvegicus*, in rural populations (enclosures) by studies of outbreaks of *Angiostrongylus cantonensis*, in two provinces, during 2008-2011 (14). Rodents are recognized as reservoirs of numerous viral, bacterial, and helminth endoparasites, which can infect humans through direct contact with feces (15).

The evaluation of rodent helminthiasis in different geographical areas will establish the impact of rodent-associated diseases on human and livestock health (16).

Developing countries have characteristics of poverty, overcrowding and lack of basic services that allow the development of large numbers of rodents that coexist with the population, which represents a greater risk for the acquisition of zoonotic diseases.

The objective of the present study was to identify intestinal helminths in wild rats (*R. rattus* and *R. norvegicus*), in five provinces of different regions to assess the distribution of parasite species present, and their relevance as health-important reservoirs public.

## MATERIALS AND METHODS

**Catches and study site.** 211 rodents were captured during January 2014 and December 2017, in five provinces (Guayas, Santa Elena, Los Ríos, Napo and Cañar), at the convenience of a research project approved in 2013 by the scientific committee, on the use of facilities,

handling of reagents and the animals used (supervised by the head of the Bioterio), on definitive hosts of *A. cantonensis* in endemic areas of Ecuador. It was then decided to record the presence of other gastrointestinal parasites.

**Field work.** The sites were strategically chosen, according to the cited literature (14) and new places were included: Guayas (cantons: Guayaquil and Duran), Los Ríos (Babahoyo canton), Cañar (La Troncal canton), Napo (Tena canton) and Santa Elena (Cadeate campus). Nine live capture Tomahawk traps (15×15×45 cm) were used for rats with the help of non-toxic baits (bread, sausages, chicken and cheese). The provenance and physical parameters (total length, tail length, feet and ears, and weight) were established to establish the species (17,18).

**Collection and processing of samples.** The procedures for the capture, transfer and handling of live animals were prepared in the facilities of the Bioterio Platform of the National Institute of Public Health Research "Dr. Leopoldo Izquieta Pérez" in the city of Guayaquil, at a temperature of 20-25°C, with an average relative humidity of 40-70% and a light / dark photoperiod of 12/12 hours, maintaining free access to water and food. Later they were registered (number/origin/sex) and weighed.

Animals were managed according to the guidelines stipulated in the Manual on the Care and Use of Experimental Animals, Chapters: II, III, V (Literals: G, H), IX, X, XI, XII.

Likewise, the universal rights of animals were respected in accordance with the Declaration of the World Medical Association on the use of animals in Biomedical research (19). Likewise, the universal rights of animals were respected in accordance with the Declaration of the World Medical Association on the use of animals in Biomedical research (20).

The abdominal cavities of *Rattus* spp. were examined for parasites: cysts, larval stages, juveniles and adults, in the different organs (esophagus, liver, stomach, intestine and colon). Additionally, the presence of eggs in feces was observed by direct observation in physiological saline and/or with Lugol. The eggs should be of the embryonated or larvae type, thick or thin shell, with the presence of hooks in the scolex, or with the presence or absence of polar filaments, depending on the species (13).

For the identification of adult helminths, the nematodes were clarified in a mixture of alcohol with phenol and the flatworms were fixed with 10% formalin. The morphological details of the adult parasites and eggs were observed with the aid of a light microscope (13). All helminths were processed using standardized parasitological techniques for identification at the species level (13,21).

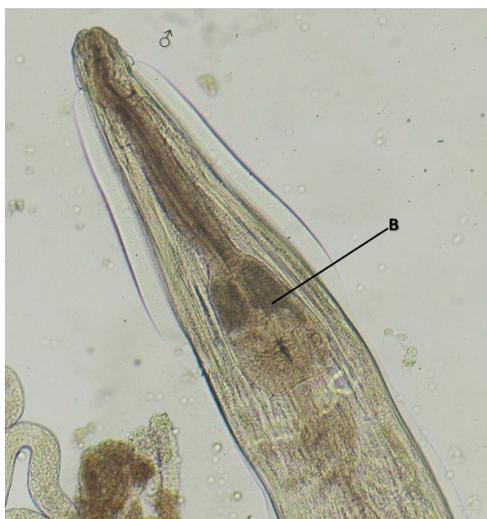
The frequencies of presentation of the parasites are expressed as a percentage. Possible associations between helminth species with species variables were evaluated by means of the chi-square test and Fisher's exact test (13) and the risk of infection was determined using the prevalence ratio (RR) evaluated. Statistics were obtained from the Epi-info 7 program (22).

## RESULTS

Of the two hundred and eleven rats captured, 125 were found parasitized with some helminth (59.2%). *R. norvegicus* 112/191 (58.6%) and *R. rattus* 13/20 (65%). *N. brasiliensis*, *H. spumosa*, *H. diminuta*, *H. nana*, *C. fasciolaris* and *M. moniliformis* were identified (Figures 1,2,3,4,5,6).



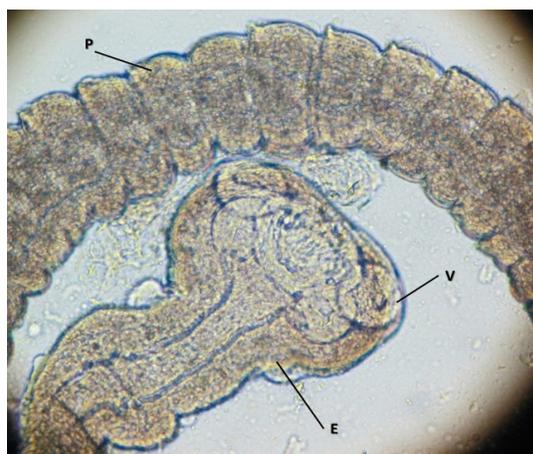
**Figura 1.** *Nippostrongylus brasiliensis* adulto mancho, con espículas (s).



**Figura 2.** *Heterakis spumosa* adulto macho, vista anterior bulbo esofágico (B).



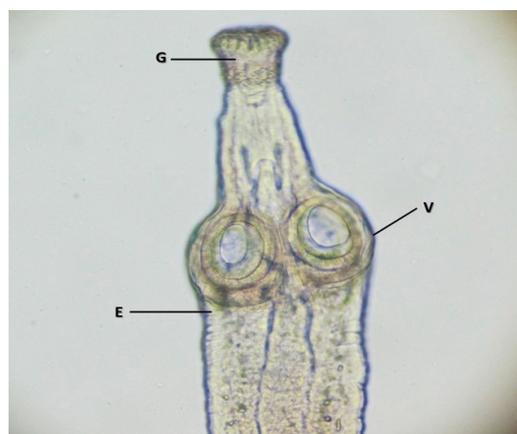
**Figura 3.** *Cisticercus fasciolaris* larva L1, corona de ganchos (G) en el escólex.



**Figura 4.** *Hymenolepis diminuta* adulta, escólex (E) con ventosas (V), y proglotides (P).



**Figura 5.** *Hymenolepis nana* adulta, escólex (E) con ventosas (V) y corona de ganchos (G).



**Figura 6.** *Hymenolepis nana* adulta, escólex (E) con ventosas (V) y corona de ganchos (G).

Prevalence Ratio test (RR): 1.7 (*H. diminuta*) and 1.785 and Fischer's test: 0.5211 (*H. diminuta*) and 0.1137 (Table 1).

Six species of helminths were found in *R. norvegicus*, two nematodes (*N. brasiliensis* 112 rodents and *H. spumosa* in three individuals) and four cestodes (*H. diminuta* in 28 rodents, *H. nana* in 10 rodents, *M. moniliformis* in 5 rodents and *C. fasciolaris* in 21 rodents). The Prevalence Ratio values: -1, 1.72, 1.94, 1.77, 1.86, 3.62 respectively. Fischer test: 0.26 (*H. spumosa*), 0.078 (*M. moniliformis*) and 0.0058 (*H. nana*), the rest of parasites with zero value (Table 2).

In the Province of Guayas, 20 *R. rattus* and 165 *R. norvegicus* were captured. While, in Santa Elena, Los Ríos Cañar and Napo only *R. norvegicus* were captured: 10, 3, 2 and 2 respectively. In Guayas it was captured and found a greater number of parasites than the other provinces (Figure 7; Table 3).

**Table 1.** Helminth fauna found in black rats (*Rattus rattus*). Ecuador.

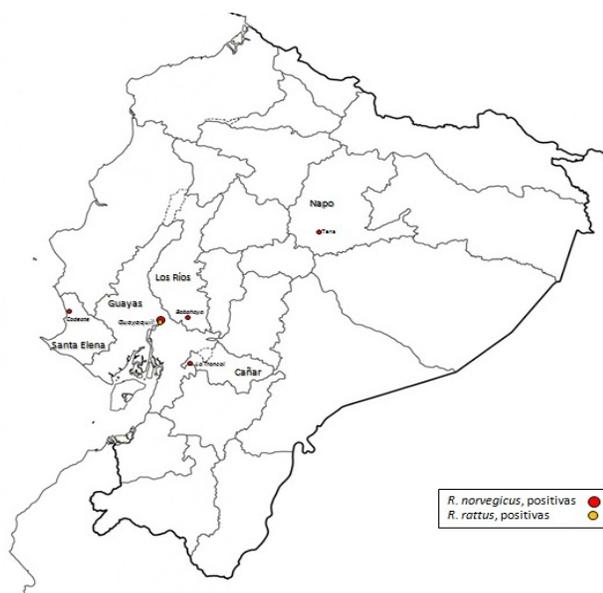
Parasite	# <i>Rattus</i> spp	Infected Rats	Parasite Prevalence	Number of Parasites	Relative Risk (RR)	p Fisher exact
<b>Cestodes</b>						
<i>Hymenolepis diminuta</i>	20	3	15%	11	1.7	0.5211
<i>Cysticercus fasciolaris</i> .	20	5	25%	7	1.875	0.1137
<b>Nematodes</b>						
<i>Nippostrongylus brasiliensis</i>	20	5	25%	121	1.875	0.1137

**Table 2.** Helminth fauna found in brown rats (*Rattus norvegicus*). Ecuador.

Parasite	# <i>Rattus</i> spp	Infected Rats	Parasite Prevalence	Number of Parasites	Relative Risk (RR)	p Fisher exact
<b>Cestodes</b>						
<i>Hymenolepis diminuta</i>	191	28	15%	135	1.94	0
<i>Hymenolepis nana</i>	191	10	5%	125	1.774	0.0058
<i>Cysticercus fasciolaris</i> .	191	21	11%	53	1.868	0
<i>Monofiliformis moniliformis</i>	191	5	3%	6	3.621	0.078
<b>Nematodes</b>						
<i>Nippostrongylus brasiliensis</i>	191	112	59%	2519	-1	0
<i>Heterakis spumosa</i>	191	3	2%	106	1.7248	0.2686

**Table 3.** Helminth fauna *Rattus* spp. according the selected provinces. Ecuador.

Provinces/ Parasites	<i>Hymenolepis diminuta</i>	<i>Hymenolepis nana</i>	<i>Cisticercus Fasciolaris</i> .	<i>Moniliformis moniliformis</i>	<i>Nippostrongylus brasiliensis</i>	<i>Heterakis spumosa</i>
Guayas	27	10	39	10	73	3
Santa Elena	-	-	-	-	2	-
Los Ríos	-	-	-	-	1	-
La Troncal	1	-	-	-	5	-
Napo	-	-	-	-	2	-
<b>Total</b>	28	10	39	10	83	3

**Figure 7.** Political map of Ecuador with reference points of where the rodents (*Rattus* spp.) with gastrointestinal parasites were captured.

Additionally, six *Filaria* spp. Larvae (unidentified) were found inside the coronary artery tissue of four *R. norvegicus*.

## DISCUSSION

In a study carried out between 2009-2013 in different regions of the Netherlands, 40 *R. rattus* and 117 *R. norvegicus* were captured, with nine species of endoparasites, including: *N. brasiliensis*, *H. spumosa*, *H. diminuta* and *H. nana* (23).

Between 2010-2011 in São Gonçalo, Rio de Janeiro, Brazil, 114 *R. norvegicus* were trapped, only two rats were free of infection. A total of 14.401 helminths were found in the small and large intestine. About 98.7% were nematodes, including *N. brasiliensis* (11,518) and *H. spumosa* (189) and two species of cestodes: *H. nana* and *M. moniliformis* (24).

In our study of 211 rats, 124 were found parasitized with some helminth. *R. norvegicus* 112/191 (58.6) and *R. rattus* 13/20 (65%), lower values were found in other countries.

Although the helminth species found in *R. norvegicus* are similar worldwide, the structure of the helminth community differs according to regional characteristics (for example, air temperature, soil pH, precipitation, and presence of the intermediate host). In addition, previous studies have shown that nematodes and cestodes are more abundant in *R. norvegicus* communities (24).

The most prevalent helminth was *N. brasiliensis* (25% for *R. rattus* and 53% *R. norvegicus*) and abundant (2519 organisms) in this study. This nematode has already been reported before in *Rattus* spp., in other countries in South America, due to the fact that it acquires the parasites by oral ingestion and penetration into the skin of infectious larvae (L3), which is the most common means (24).

Studies conducted in Brazil have shown that when young rats are exposed to infection by *N. brasiliensis*, helminth persists into adulthood and young rats do not cause expulsion of the parasite, whereas adult rats exposed to *N. brasiliensis* they develop an immune response. Based on this fact, the infection of *R. norvegicus* by *N. brasiliensis* observed in the present study may have occurred during juvenile age, which favors an increase in parasite load over time. Transmission may also be related to the size of the host group, the size of the host's field of origin, and the degree of sociability of the host (24).

*Hymenolepis* spp., was the second most prevalent helminth in this study (13.3% *H. diminuta* and 4% *H. nana* in *R. norvegicus*, and 15% *H. diminuta* in *R. rattus*). Previous studies of cestodes have reported higher numbers. Both *H. nana* and *H. diminuta* are zoonotic cestode helminths, although the latter is rarely seen in humans (13,23,24). *H. diminuta* is transmitted to humans by ingestion of *Tribolium confusum* (flour beetle as an intermediate host) with infested cereals, or via the fecal-oral route. *H. nana* is transmitted through fecal-oral contact (eggs), or by accidental ingestion of intermediate hosts (arthropods) that harbor cysticercoids (25). Infections in humans by these two species are mostly asymptomatic, although they can produce weakness, headache, abdominal pain, and diarrhea (23,25).

A species of helminth transmitted by ingestion of eggs in the soil is *H. spumosa* (only 1.5% of infected *R. norvegicus*). Similar studies in South America report rats (*Rattus* spp.) high infection rates (13,23,26) that can be acquired in underground burrows, which harbor the parasite (26).

*M. moniliformis*, with a lower prevalence than in other studies (2.4%), is a parasite of public health interest because there are reports of infection in humans when ingesting the infected intermediate host, which are arthropods such as cockroaches (24), causing gastrointestinal disturbances, especially in children, who live in conditions of inadequate hygiene, without sanitary facilities (24, 26).

Another helminth present in the study was *C. fasciolaris* (9.9% in *R. norvegicus*) lower than other studies carried out in South Korea, where they were found in 287 rats, 97 (33.8%) infected with this parasite (27), corresponds to larval stage of the *T. taeniaeformis* cestode. The eggs released from the proglottids mature, exit with the feces and when ingested by a susceptible animal, are activated during passage through the stomach and the larvae (oncospheres) migrate to the liver, where they continue to develop into a cyst (28). This helminth has been documented in human cases in previous studies (29).

The most commonly recognized zoonotic parasite in the present study was *H. diminuta*, with the highest infestation rate in *R. norvegicus* followed by *H. nana*, *C. fasciolaris*, and *M. moniliformis*. In these rodents it is of great importance since they constitute a permanent reservoir for the transmission of diseases to humans.

Studies in Lima, Peru showed a statistical association between infection with this parasite (*H. Diminuta*) and the category of rats (*R. rattus* greater and less than 20cm) with a value of  $p=0.021$ , concluding that there is no statistical association between some other worm of zoonotic interest and the variables under study in this study (13). In the same way, in our study an association was found between the infection (*H. diminuta*) for *R. rattus*, with a  $p:0.52$  and for *R. norvegicus* (with *M. moniliformis*) with  $p:0.078$ . Because the number is less than in relation to the aforementioned study.

Overcrowding and poor sanitation practices in urban areas expose inhabitants to the risk of infection. Although the species of medical importance were less abundant in this study, we noted the need for continuous monitoring of

rat populations, new research into the ecology of diseases, and health education programs to reduce human transmission of intestinal parasites (26).

In conclusion, considerable differences have been shown in the variation of helminth species between: *R. rattus* and *R. norvegicus*. The variation of helminth species found in the intestine (four species) and parasitic infections in other organs (livers and coronary arteries) were recorded more in brown rats than in black rats, the first, in the same period, had the highest number of simultaneous infections, compared to black rats in the same environment.

Finally, the rodents studied harbored several parasitic parasites, taking measures to control

the rodent population and advising the local population on the risk of disease transmission through rodents is recommended.

### Interest conflict

None declared.

### Acknowledgement

To all the members of the National Reference Center for Parasitology of the National Institute of Public Health Research (INSPI) "Leopoldo Izquieta Pérez". Guayaquil, Ecuador. To Dr. María Beltrán, Manuel Tantaleán and members of the Department of Parasitology of the National Institute of Health of Peru. And Mrs. Elsa P. Amador Palma for the capture method used.

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