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Identificación de *Angiostrongylus* spp en *Lissachatina fulica* y *Cornu aspersum* en Antioquia, Colombia

***Angiostrongylus* spp. en *L. fulica* y *C. aspersum* en Colombia**

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Janeth Pérez-García: Conceptualization, data curation, investigation, methodology, formal analysis visualization, project administration and writing.

Introduction. Abdominal and neural angiostrongyliasis caused by *Angiostrongylus costaricensis* and *A. cantonensis,* respectively, are zoonotic diseases involving snails as intermediate hosts. Colombia has reported human cases and the increasing distribution of *Lissachatina fulica* and *Cornu aspersum* raises public health concerns due to potential risk of disease transmission in areas where parasites and hosts coexist.

Objective. To identify the presence of *Angiostrongylus* spp. in snail species *L. fulica* and *C. aspersum* in the region of Antioquia, Colombia.

Materials and methods. This prospective cross-sectional study had a population of 5,855 *L. fulica* and *C. aspersum* snails captured in the ten cities of Valle de Aburrá (Antioquia, Colombia) 169 samples were collected in 28 sampling points. Lung tissues of the collected snails were dissected and analyzed to detect *Angiostrongylus sp.* through molecular techniques.

Results. *Angiostrongylus* sp. were identified in both *L. fulica* and *C. aspersum. A. costaricensis* was detected in 18 pooled samples (30%; 95% CI 19.2%-43.3%), and Medellín was the municipality with the highest number of positives (33.3%). 72.2% of positive places report presence of rodents. None of the tests were positive for *A. cantonensis*.

Conclusion. Our findings provide important insights into the epidemiology and distribution of *Angiostrongylus* spp. in the region of Antioquia, Colombia. The identification of these parasitic nematodes in *L. fulica* and *C. aspersum* highlights the potential role of these snails as intermediate hosts in the transmission of *Angiostrongylus* infections in Valle de Aburrá, with implications for both human and veterinary health.

Keywords: *Angiostrongylus*; Strongylida infections; snails; Mollusca; zoonoses; Colombia.

Introducción. La angiostrongiliasis abdominal y neural, causadas por *Angiostrongylus costaricensis* y *A. cantonensis,* respectivamente, son zoonosis que involucran caracoles como hospederos intermediarios. Colombia ha reportado casos en humanos y el aumento en la distribución de *Lissachatina fulica* y *Cornu aspersum* aumenta la preocupación en salud pública debido al riesgo potencial de transmisión en áreas donde parásitos y hospederos coexisten.

Objetivo. Identificar la presencia de *Angiostrongylus spp.* en caracoles *L. fulica* y *C. aspersum* en una región de Antioquia, Colombia.

Materiales y métodos. Estudio transversal prospectivo con una población de 5.855 caracoles *L. fulica* y *C. aspersum* capturados en diez ciudades del Valle de Aburrá;169 muestras fueron recolectadas en 28 puntos de muestreo. Tejidos pulmonares de los caracoles fueron disecados y se emplearon técnicas moleculares para detectar *Angiostrongylus spp.*

Resultados. *Angiostrongylus costaricensis* fue detectado en 18 muestras agrupadas (30%; IC 95% 19,2%-43,3%), tanto en *L. fulica* como en *C. aspersum*. Medellín fue el municipio con mayor número de muestras positivas (33,3%). El 72,2% de los lugares positivos reportaron la presencia de roedores. Ninguna de las pruebas fue positiva para *A. cantonensis.*

Conclusión. estos hallazgos brindan información importante sobre la distribución de *Angiostrongylus spp.* en la región de Antioquia, Colombia. La identificación de estos parásitos nemátodos en *L. fulica* y *C. aspersum* resalta el potencial papel de estos caracoles como hospedadores intermediarios en la transmisión de infecciones por *Angiostrongylus*, en el Valle de Aburrá, con implicaciones para la salud humana y veterinaria.

Palabras clave: *Angiostrongylus*; infecciones por *Strongylida*; caracoles; moluscos; zoonosis; Colombia.

Angiostrongylus spp are nematodes infecting humans and animals. These parasites are commonly found in rodents but can also reside in snails like *Cornu aspersum* (common garden snail) and *Lissachatina fulica* (giant African land snail) (1). *Angiostrongylus sp.* are endemic to Asia, the Pacific Islands (Hawaii, Vanuatu, or Thailand), Australia and the Caribbean islands. These parasites cause zoonotic diseases in humans: abdominal angiostrongyliasis caused by *A. costaricensis,* and neural angiostrongyliasis caused by *A. cantonensis*. Recent human cases of the disease have been reported in various countries such as the United States, Spain, Ecuador, Brazil, Martinique (French west Indies), Venezuela and Colombia (1-5). The first case of human infection in Colombia was detected in 1979, with a total of eight events were reported by 2020 (6).

The parasite life cycle begins with rats as definitive hosts ingesting it. Then it penetrates the digestive system, enters the bloodstream, and migrates to the central nervous system until the parasite reaches the sub-adult stage. Finally, it migrates to the pulmonary arteries, depositing eggs that migrate to the lungs. In *A. costaricensis,* oviposition and hatching occur in the ileum. Hatched immature larvae migrate to the trachea, get ingested, and pass through the digestive system before being excreted. Snails become intermediate hosts by ingesting fecal material from infected rats containing first stage larvae (L1) of *Angiostrongylus spp*. Within the snail, the parasite reaches its final infective stage known as L3. A new rat ingesting the infected snail allows the infective larvae to migrate via the bloodstream and initiate a new cycle. Humans become accidental hosts through the consumption of raw or undercooked snails or slugs (7). Additionally, *A. costaricensis* can infect dogs and make them possible parasite reservoirs (8,9).

The giant African snail has been reported in more than 70% of the departments of Colombia, in less than a decade, particularly in urban areas, where it finds refuge (10-12). In 2017, Cordoba et al. reported the presence of three parasitic nematode genera (*Angiostrongylus, Aelurostrongylus and Strongyluris*) in giant African snails collected from nine cities of Valle del Cauca (Colombia): Jamundí, Cali, Palmira, Buga, Tuluá, Bugalagrande, Cartago, Dagua and Buenaventura. However, they did not confirm the specific presence of *A. cantonensis* (6,13,14).

Information on the prevalence of these parasites in snail intermediate hosts is scarce in Colombia. The transmission of the disease to humans is presented through handling the animal and fomites contaminated with snail secretions. Therefore, the identification of *Angiostrongylus* circulating in the environment would determine the risk and would allow the establishment of efficient protection and control measures of both the disease and its intermediate snail host. While current Colombian regulations aim to control the African snail population (15), the recognition of the parasite will allow the establishment of more radical control measures and reduce the risk of human infections. The aim of this study was to assess the presence of *Angiostrongylus spp*. in *C. aspersum* and *L. fulica* within the metropolitan area from Valle de Aburrá during 2022.

Materials and methods

Type of study and sample size

Prospective, cross-sectional, descriptive design. Data were collected from 28 sampling points distributed in ten cities of the metropolitan area from Valle de Aburrá (Antioquia, Colombia): Barbosa, Bello, Caldas, Copacabana, Envigado, Girardota, Itagüí, La Estrella, Medellín, and Sabaneta. Due to resources limitations, a convenience sample size was included.

Area of study: climate and orography

Valle de Aburrá exhibits significant altitudinal variation, covers varying altitudes, with its major cities positioned between approximately 1,400 meters (4,593 feet) and 1,600 meters (5,249 feet) above sea level. The region has a warm climate with relatively stable year-round temperatures ranging from 18° C to 28° C. Rainfall follows a bimodal pattern with a wet season from April to November and a drier season from December to March.

This region encompasses a diverse range of urban and rural environments, each with unique climatic characteristics. Barbosa, situated in the northern region, is surrounded by moderate rainfall, and rolling terrain. Girardota, a primarily rural area undergoing recent urbanization, receives high precipitation and has hilly topography. Itagüí, characterized by warm and humid conditions, exhibits variation in elevation. Bello, also situated to the south, shares similar condition to Itagüí. Copacabana experiences a tropical climate with a well-defined wet and dry season. To the South, Envigado features a warm and temperate climate. La Estrella, also to the South, is characterized by flat terrain and a warm, moderately rainy climate. Caldas, a predominantly residential area in the Southwest, possesses flat terrain and a moderate climate. Sabaneta, features a warm and temperate climate. Lastly, Medellín, the largest city in the region resides within a mountain enclosed valley, resulting in a subtropical mountainous climate with moderate temperatures.

Snail sampling and specimen preparation

Sampling sites were chosen based on reports from the local community. Collection and identification of the species were carried out by the environmental authorities (Área Metropolitana del Valle de Aburrá). The collection period was from October 2022 to January 2023. Animals were transported to Parque de la Conservación, where they were anesthetized with 5% ethanol, followed by euthanasia via immersion in 95% ethanol according to the recommendations of the Guide for Euthanasia of animals of the American Veterinary Medical Association 2020 (16). The shells were separated from the soft tissues. For small individuals, the entire tissue was collected. And for larger snails, a portion of the mantle, foot and lungs were obtained. A total of 10 gr were obtained per animal. Samples were placed in screw-cap urine cytochemical jars, kept refrigerated at approximately 4° C, and transported daily to the laboratory at Instituto Colombiano de Medicina Tropical (Sabaneta, Colombia). There the samples were preserved at -20° C for DNA extraction.

DNA extraction

The extraction of 200 µg of the samples was performed with the Qiagen DNeasy Blood and Tissue Spin Column Kit®, following the manufacturer's protocol. Tissue was homogenized in 24 ml of lysis buffer (0.1M Tris - =.1 M EDTA - 0.01 M NaCl - 0.5% sodium dodecyl sulfate) for each gram of macerated tissue, combined with six to eight beads for 3 minutes. 150 µg of proteinase K was included after incubation at 56° C for 1 to 48 hours, vortexing every hour until a homogeneous solution was obtained.

Molecular testing

DNA quantification was performed with a multiple real-time PCR (qPCR) for identification of genus *Angiostrongylus* sp. The forward primer of AcanITS1F (5'-TTC ATG GAT GGC GAA CTG ATA G-3') and AcanITS1R (5'-GCG CCC ATT GAA ACA TTA TAC TT-3') were selected to amplify a fragment that was detected by the probe, AcanP (6-FAM-ATC GCA TAT CTA CTA TAC GCA TGT GAC ACC TG-BHQ1). The PCR tests were carried out in a thermal profile that consisted of a reverse transcription step at 50° C for 2 minutes, followed by 40 cycles of amplification with initial denaturation at 95° C for 30 seconds, alignment and extension at 60 °C for 30 seconds, finalizing with 2 min at 56°C.

Statistical analysis

The data were explored using the R program. Graphs and descriptive measures were generated for each variable: snail species, city, area type, presence of snails at the sampling site and presence of rodents at the sampling site. The chi-square test was calculated as a measure of association or Fisher's test when necessary. A Pearson correlation test was performed to determine the relationship between positivity and the number of positive points by geographic location. The frequency of positivity was calculated with a 95% confidence interval. Statistical significance was set at a p-value of less than 0.05.

Ethical Considerations

The project was approved by the Institutional Committee for the Use and Care of Animals (CICUA) of the CES University (approved by means of minutes number 27 of December 14, 2021, with project Code 65) and the Ethics Committee of the

Parque de la Conservación (approved by means of minutes number 2 of November 30, 2021).

Results

Characteristics of snail sampling sites

Due to limited financial resources only 169 individuals were sampled at 28 sampling points. Distribution of these points were: Medellín (28.57%, n= 8 points), Bello (14.28%, n=4 points), Barbosa, Copacabana, Girardota, and Itagüí (10.71%, n=3 points each city) and Caldas, Envigado, Sabaneta, and La Estrella (3.57%, n= 1 point each city). The distribution by species collected in each of the cities is presented in figure 1.

Each sampling point yielded individuals of only one species. *L. fulica (75%,* n=21 points) was the dominant species, followed by *C. aspersum* (25%, n=7 points). Most of the sampling points (78.57%, n=22 points) were in urban areas. There was no statistically significant association between area type (urban vs. rural) and the number of snails observed (p-value = 0.2300). Ten points (35.71%) presented a low quantity of snails, while the remaining eight points (28.57%) were characterized by high abundance.

Most samples were collected from urban areas, as shown in figure 2. No specimens of *L. fulica* were collected in rural areas. There was no statistically significant association between the collected species and the type of area (p-value = 0.2875). Of the places where *L. fulica* was captured, 80.95% (n=17) reported the presence of rodents, compared to only 42.85% (n=3) of the sites where *C. aspersum* was collected. However, there was no association between the collected species and the presence of rodents (p-value = 0.1473).

Presence of angiostrongyliasis

A total of 60 qPCR test were performed by *pooling* two to three individuals per sample of the 169 animals. The proportion of pools positive for *A. costaricensis* was 30% (95% CI, 19.2%-43.3%), corresponding to 18 positive pools. None of the samples tested were positive for *A. cantonensis.* Of the sites with *C. aspersum*, 46.15% (n=6 points) were positive, while 53.84% (n=7 points) of the sites with *L. fulica* were positive for *A. costaricensis.* This difference between snail species and study site was statistically significant (p-value = 0.0286).

In addition, some environmental factors potentially associated with the presence of *A. costaricensis*-positive snails revealed no statistically significant associations, as shown in table 1.

Medellin had the highest number of positive samples (33.3%, n=6). Of all the individuals, the highest number of positive samples originated from urban areas (77.77%, n=14) and from places with a low number of snails observed at the time of collection. It should be noted that the presence of rodents in the sampling sites and positive results in snails for *A. costaricensis* was 72% of all the sites included in the study (n=13). Finally, no correlation was found between the number of snails collected and the number of sampling sites positive for *A. costaricensis* (p-value = 0.4408). The geographical distribution of snails infected with *A. costaricensis* in the metropolitan area from Valle de Aburrá is represented in figure 3.

Discussion

This study found 30% positive pooled samples of *Angiostrongylus* sp in snails across the ten cities of the metropolitan area from Valle de Aburrá. This value is within the broad range of prevalence reported in mollusks across various geographical locations such as Brazil, Ecuador, Egypt, and China. which can vary from 0.2% to 93.4%, (3,17-19). These countries might share similarities influencing the transmission of *Angiostrongylus sp.,* such as climate variability, ecological diversity, and availability of host.

In Colombia, few studies have described the presence of the parasite in African snails (20); Giraldo and collaborators in 2018 confirmed the presence of *A. cantonensis* in *L. fulica* the lung tissue from Buenaventura, Valle del Cauca, using qPCR (12). This method is considered as a direct test of the presence and should be used for parasite detection throughout the transmission cycle, and through this study it was possible to standardize it for continuous active epidemiological surveillance of the parasite.

The prevalence of angiostrongyliasis or the presence of *Angiostrongylus spp* in human population in Antioquia, in intermediate hosts or in final hosts such as rodents, remains unknown. A study conducted in different departments of Colombia in 2019 reported a 3.9% frequency of *Angiostrongylus vasorum* in *L. fulica* but excluded the cities in this study and did not describe the presence of *A. costaricensis* (21). The presence of this parasite should be considered as an epidemiological alert for health authorities, considering transmission routes as ingestion of contaminated vegetables, fruits, or legumes with *Angiostrongylus* spp. Recognized cases of the disease are scarce in Colombia, *A. costaricensis* has been reported in departments like Caquetá, Huila, Putumayo, Tolima, Valle, and Vaupés, but none reported for the studied cities (14). This study aimed to determine the presence of *Angiostrongylus* spp in *C. aspersum* and *L. fulica* in the ten cities of the metropolitan area from Valle de Aburrá.

The statistically significant difference in infection rates and the municipality of collection, despite low sample sizes per municipality, shows the need for more in depth analysis of parasite prevalence across the metropolitan area from Valle de Aburrá due to its potential public health concern. Additionally, the higher number of *L. fulica* collected in urban areas reflects environmental awareness programs that have been carried out by local authorities but may not accurately represent the actual distribution of both species in the territory.

The reported association between the self-reported presence of rodents and positive snails suggests that the disease cycle may be in an enzootic phase. However, the imminent risk could be determined by incorporating rodents into the surveillance program of the health impact of exotic snails in Valle de Aburrá, as well as studies of abundance of both snail species and prevalence through systematic sampling of angiostrongyliasis by municipality. These studies would provide more accurate data and identify risk characteristics at specific sites.

Future studies should further investigate into the eco-epidemiological role of snails, rats, the parasite, and human communities in proximity. The epidemiology of angiostrongyliasis is complex and requires recognizing risk factors associated with the geographical conditions where *Angiostrongylus* has been identified for implementing effective measures for the prevention of human cases (22). This is crucial in Valle de Aburrá because of high fauna biodiversity, microecosystems, water sources, land use patterns and human settlements, all of which can be different in each city.

This is the first report of *A. costaricensis* in snails from seven of the ten cities of the Valle de Aburrá in Colombia. These findings should be considered as an epidemiological alert by the authorities to adapt protocols for managing and preventing infestation by invasive species like the African snail *L. fulica,* and the subsequent transmission of disease-causing parasites to humans.

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Conflicts of interest

The authors declare that they do not have any conflicts of interest.

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Table 1. Characteristics of study individuals according to the presence of *Angiostrongylus costaricensis* by qPCR. Valle de Aburrá (Antioquia, 2021).

	Positive		Negative		*p-value*
Variable	**n**	**%**	**n**	**%**	
Species					
Lissachatina fulica	9	50.00	31	73.80	0.1352
Cornu aspersum	9	50.00	11	26.19	
City					
Barbosa	3	16.67	3	7.14	0.4003*
Bello	3	16.67	5	11.90	
Caldas	2	11.11	1	2.38	
Copacabana	0	0.00	6	14.29	
Envigado	0	0.00	2	4.76	
Girardota	1	5.56	5	11.90	
Itagüí	1	5.56	5	11.90	
La Estrella	0	0.00	3	7.14	
Medellín	6	33.33	8	19.05	
Sabaneta	2	1111	4	9.52	
Zone					
Urban	14	77.77	34	80.95	0.7400*
Rural	4	22.22	8	19.04	
Presence of snails at sampling site					
High	4	22.22	14	33.33	0.6250*
Medium	6	33.33	10	23.81	
Low	7	38.88	17	40.47	
Accidental	1	5.55	1	2.38	
Presence of rodent at sampling site					
Yes	13	72.22	27	64.28	0.7659*
No	5	27.77	15	35.71	
Total	18	100%	42	100%	

*Fisher exact test.

Figure1. Number of samples by municipality. Valle de Aburrá (Antioquia).

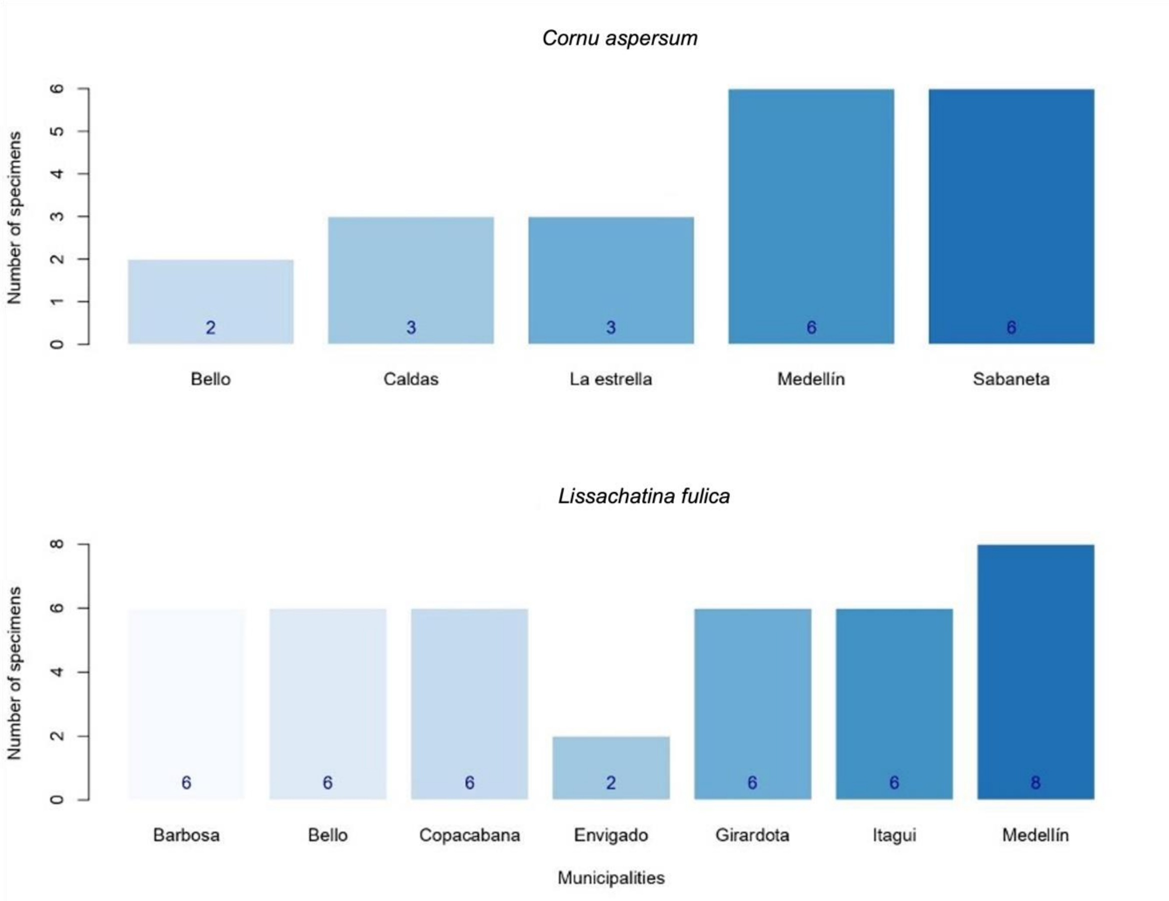

Figure 2. Distribution of the individuals included in the study by species and collection area. Valle de Aburrá (Antioquia).

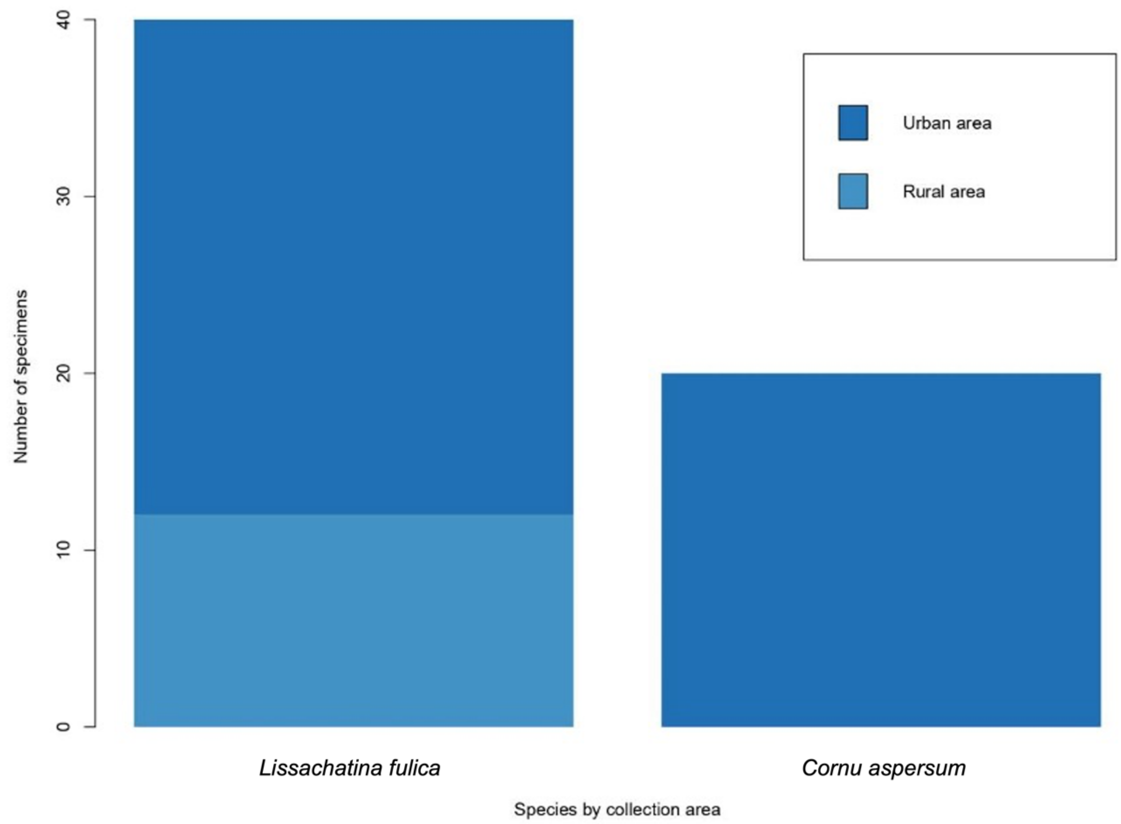

Figure 3. Geographical distribution of snails with *A. costaricensis.* Valle de Aburrá (Antioquia).