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Presence of Achatina fulica in Presidente Franco, near the Triple Border, Paraguayan side: a parasitological screening

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Abstract: *Achatina fulica* (synonym. *Lissachatina fulica*) or commonly the African Giant Snail (AGS) appears among these exotic species invaders, originating from the East of Africa, and was introduced voluntarily or accidentally in more than 50 countries, which harmed biodiversity, ecosystems, agriculture, and public health. This study aimed to confirm the presence of *Achatina fulica* in Paraguay and analyze feces, slime, and lung tissue in searching for eggs, cysts, and larvae of parasites. Fifty specimens were captured in the city of Presidente Franco, Paraguay. The presence of parasites was evaluated using routine parasitology techniques. The average weight was 21.61 grams and 6.26 cm in length. 93% (46/50) of the specimens had parasite eggs, larvae, and cysts in fecal samples. No eggs or larvae were found in the slime. In the analysis of the lung tissue, eggs and larvae of Strongylid type (n=135) and Ascaris (n=5) type were observed. AGS represents a potential threat to the area's biodiversity and is considered a carrier of parasites. More studies at the morphological and molecular level of the larvae recovered are expected to be carried out in the future.

Keywords: African snail, zoonotic, gastrointestinal parasites, slime, lungs.

1. Introduction

Achatina fulica (synonym. *Lissachatina fulica.)* spread throughout the world outside its native range since the 19th century; this species was first introduced to Madagascar and Mauritius. In the 1900s, it was introduced to South America and the United States (OIRSA, 2020). The spread of the African snail could have been due to intentional (e.g., food, pets, esoteric or religious rituals) or accidental transportation (e.g., cargo). Currently, it is found in Africa, the Americas, South and East Asia, Oceania, and certain areas of Europe (Sridhar et al., 2014).

Helminth parasites rank high on the World Health Organization's list of neglected diseases, accounting for eight of the 20 neglected diseases in public campaigns (Schneider et al., 2011). The presence of *A. fulica* throughout an entire territory can also be seen as a serious public health problem because they are intermediate hosts of parasitic helminths that can affect men, animals, and plants by ingesting plants, fruits with the presence of these mollusks or contaminated by their excrement, among them, *A. cantonensis and A. costaricensis, Aelurostrongylus abstrusus, Rhabditis* spp., *Strongyluris spp.* (Chen et al., 2011; Muzzio, 2011; Penagos-Tabares et al., 2019). Helminth zoonoses are constantly changing regarding host range, geographic distribution, and the emergence of new or unexpected challenges. Another public health problem that experts are calling for is the shells of dead organisms since, when filled with rainwater, they can harbor populations of vectors of diseases such as Dengue, Yellow fever, Zika, and Chikungunya. However, attention must be paid to the species, given the risk of disease transmission to humans and other higher vertebrates (Andreazzi et al., 2017).

The presence of *A. fulica* has been increasingly reported in the local press (ABC Color 2019; Norte Chaco 2023; Última Hora 2023) and informally by people who claim to have this snail in their yards, especially in the Triple Border area in Paraguay. This study aimed to document the presence of *A. fulica* and to analyze feces, slime, and tissues looking for eggs, cysts, and larvae, aimed to reveal a local scenario in terms of parasitology that can expand the knowledge of this invasive species and serve for future research projects.

2. Materials and Methods

2.1. Study area

This study was carried out in Presidente Franco (-25.571194, -54.662855), which belongs to the Department of Alto Paraná, Paraguay (Figure 1). It is known as the city of the Three Borders and the Monday Falls since the territories of Puerto Iguazú, Argentina, and Foz de Iguazú, Brazil, converge. Presidente Franco has an area of 122 Km^2 and a population of 88,744 inhabitants (833,77 hab./km^2) (https://www.ine.gov.py/censo2022). The choice of the collection site was based on citizen reports and local media that warned about an invasion of African snails in the department of Alto Paraná.

Figure 1 – Map of Paraguay and borders, and the Alto Parana Department (https://www.geologiadelparaguay.com.py) (red region). Mapas Geológicos Departamentales del Paraguay (2021). Collection site of President Franco (black circle).

2.2. Animal collection, identification, and classification

Fifty specimens of *Achatina fulica* were captured alive. The specimens were placed in a bag and a conservation container and immediately taken to the Multidisciplinary Center of Technological Research (CEMIT) to identify the species. Snails were identified according to their shape, size, markings, color, spire angle, sculpture, and aperture form (Raut and Barker, 2002). The snails were categorized into three groups by size: 1) small (3 cm shell), 2) medium (3.1 to 5.0 cm), and 3) large (5.1 cm) (Fischer and Colley, 2005) and by weight 1) small (0.1 to 20 g), medium (20.1 to 30 g), large (30.1 to >60.0 g).

2.3. Parasitological procedures

The snail's shells were cracked, and the internal parts were separated into the Stomach, Lungs, Kidney, Mantle, Digestive gland, and foot, which were put in a separate petri dish containing ethanol 70%. The lung tissue was cut into 1 x 1 cm pieces and placed on microscope slides for observation under a stereoscope. The slime used for analysis was obtained by manually stimulating the foot gland in Petri dishes of each mollusk separately and later placed on a slide for microscopic study. Observations of fecal matter were carried out following the scheme of a) Macroscopic observation, using the stereoscope to observe adults, and b) Microscopic observation; two methods were used: a direct method with a wet mount preparation and a floatation technique using saturated salt solution (1.2 density) (Segura et al., 2023, Silva et al., 2019). The recovered parasites were identified according to identification keys based on the pharynx morphology and stoma (Franco-Acuña et al., 2009). Larvae with characteristics similar to nematodes of zoonotic interest were stored in 70% ethanol or formalin for further analysis.

2.4. Data analyses

All statistical analyses were computed using Microsoft Excel 2021 and Statistical Package for Social Sciences (SPSS) 20.0. The level of significance was determined using Chi-square analysis at $p < 0.05$.

2.5. Ethics considerations

All animal experiments were conducted according to the current national and international Bioethical Laws, and the Ethics Committee CEMIT, UNA, approved the protocol. CE -0007

3. Results

3.1. Snail species detection and Morphological analysis

Fifty specimens of *A. fulica* were captured alive; they were photographed and measured in total length (cm) and total weight (g), as shown in (Figure 2 A, B). The 50 mollusks collected corresponded to the genus and species *A. fundica*. The average weight was 21.61 grams, and the length was 6.26 cm.

Figure 2 – (A) *Achatina fulica* from Presidente Franco, Eastern Paraguay. (B). Identification and preparation of specimens.

3.2. Analysis of the samples

Parasitic taxa corresponding to the phylum Nematoda and Protozoa were found in fecal samples. The 106 larvae found correspond to the order *Strongyla, Rhabditis* spp., and free-living larvae. Eggs (n=140) with characteristics similar to *Ascaris and Strongylid types* were observed. The protozoa found correspond to cysts of *Amoeba spp*. Although free-living nematodes were retrieved in all fecal samples, no specimens were found in the slime samples. However, oxalate crystals and an abundant amount of grit, such as eggs, were observed (Figure 3).

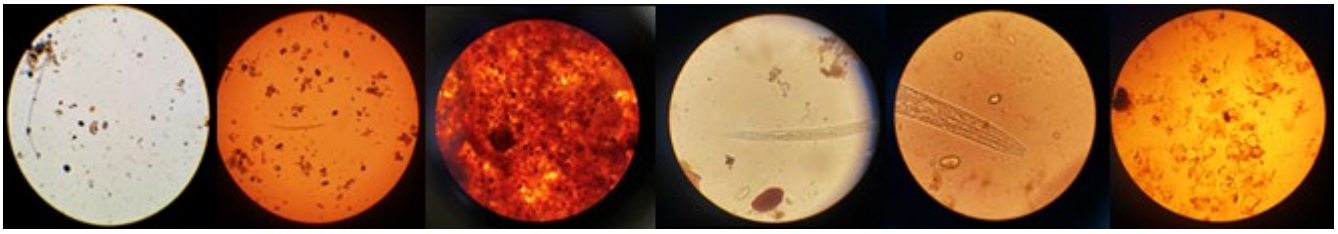

Figure 3 – Parasitological identification of eggs, larvae and crystals in *Achatina fulica* individuals from Presidente Franco, eastern Paraguay. A. Eggs in feces, B. Larvae, C. Eggs in tissue, D. Distal extremity of larvae of nematodes (*Rhabditis* spp), E. Frontal larvae of nematodes (*Rhabditis* spp). F. Oxalate crystals in slime.

3.3. Relation to size and parasite numbers

The presence of nematodes was significantly higher in mollusks above 5.1 cm ($p<0.0001$) (Table 1).

Parameters	Range	n	Eggs (Mean +SD)	Larvae (Mean +SD)
Size (cm)	0 to 3	7	2 ± 2	1 ±2
	3.1 to 5.0	3	1 ±1	1 ±2
	5.1	40	3 ±3	2 ±3
Weigth (g)	0 to 20	20	2 ±2	1± 3
	20.1 to 30	13	3±3	2 ±3
	30.1 to 60	17	4±4	3±3

Table 1 – Results of eggs and larvae of nematodes, based on weight and length, found in feces and tissue of CGA from Paraguay, expressed as mean and standard deviation.

4. Discussion

Our results suggest that the populations obtained from *A. fulica* collected in Presidente Franco are in a stable phase, presenting a variety of young and adult individuals. Regarding the size classes of *A. fulica*, it was identified that there was a higher percentage of 40 adult mollusks (> 5.1 cm), followed by three young adults (3.1 to 5.0 cm) and seven young mollusks (≤3 cm). The African snail can reach sizes of up to 30 cm in the length of the shell. However, the mean values reported are usually much smaller and vary between 4 and 10 cm (Gabetti et al., 2023; Okon and And Onwuka, 2017; Patiño-Montoya et al., 2021). Gómez-Camargo et al. (2021) mentioned that patterns in snail size can reflect the age structure of the population in a given area; it has been proposed that the establishment of *A. fulica* in new regions takes place in three stages: exponential, known as a long phase with vigorous individuals; stable, with a prevalence of a variable shell size among young and adult, and the decline phase, where young individuals are prevalent (Albuquerque et al., 2009; Gómez-Camargo et al., 2021).

The giant African land snail is an intermediate host for several parasites, including *A. abstrusus*, *Angiostrongylus cantonensis, A. costaricensis, Schistosoma mansoni, Hymenolepis* spp. as well as *Fasciola hepatica* (Hu et al., 2011; Morassutti et al., 2014; Penagos-Tabares et al., 2018; Traversa and Guglielmini, 2008; Vázquez and Sánchez, 2015). Of all the samples collected, three larvae whose morphological characteristics correspond to the metastrongylidae family were found.

Aelurostrongylus abstrusus, which is associated with *A. fulica* and other terrestrial mollusks, is medically essential for domestic animals, causing cardiorespiratory problems in felids and canids (García-Livia et al., 2023; Lopez-Osorio et al., 2021; Taubert et al., 2009). Free-living nematodes such as those of the genera *Caenorhabditis* and *Rhabditis*, which are not considered to represent a threat to the health of humans or domestic animals, have also been found in association with these mollusks. The confirmed presence of *Angiostrongyluss spp*. reported in Sao Paulo and Pernambuco, Brazil, and San Ignacio, Misiones, Argentina, in 2022 indicates the proximity of this species and the potential risk to our country. The straight distance between Presidente Franco (Alto Paraná) and San Ignacio is only 284.08 km. The natural movement of this species is 50 m per night, on average 125m per month, and 250 m *per* year (USDA–APHIS. 2005).

A common characteristic of these mollusks is the areas where they are found; they have vegetation cover, garbage, wastewater, and domestic animals. One of the determinants is humidity; the presence of sewage in the environment allows the soil to become moist even during periods of drought, which facilitates the growth and maintenance of the vegetation cover, making the places suitable for the presence of both mollusks and free-living nematodes alike *Rhabditis* spp. (Martin and Sommer, 2004; Onyishi et al., 2018; Patiño-Montoya et al., 2021; Silva et al., 2019). This species of snail often lives near human houses. As a result, contamination of the home environment through the snail's feces is possible (Pathak et al., 2023).

5. Conclusion

Achatina species can feed on the excrement of rats and other animals, such as domestic ones. For this reason, finding structures similar to *Toxocara* spp. Eggs in their feces may be due to dragging their food. Although free-living nematodes were retrieved in all fecal samples, no specimens were found in the mucus samples. However, the presence of oxalate crystals and an abundant amount of grit, such as eggs, was observed, as well as limestone rocks and walls in search of calcium supplements, which is why it is considered a species that negatively impacts the food chain. Analysis of lung tissue, larvae, eggs, and protozoa were made and collected. In future studies, we will analyze the species at the molecular level to continue the epidemiological surveillance of this invasive snail species.

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