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Research Article

Isolation and Identification of Some Pathogens of Terrestrial Snail *Rumina decollata* from Erbil Province, Kurdistan Region, Iraq

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ABSTRACT

Terrestrial snails are known to act as intermediate hosts for parasites and carriers of various bacteria. The present study aimed to isolate and identify some bacteria and larval trematodes from the terrestrial snail *Rumina decollata* (Linnaeus, 1758) collected from different locations in Erbil Province, Kurdistan Region, Iraq. Snail specimens were processed using standard laboratory techniques for bacteriological culture and parasitological examination. Several bacterial isolates were identified based on morphological and biochemical characteristics, including (*Klebsiella pneumoniae*, *Citrobacter koseri* [*Citrobacter diversus*], *Escherichia coli*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Acinetobacter baumannii*, *Citrobacter freundii*, *Stenotrophomonas maltophilia*, *Pseudomonas mendocina*, *Pantoea agglomerans* [*Enterobacter agglomerans*], *Acinetobacter calcoaceticus*). In addition, larval trematodes of *Lutztrema attenuatum* were detected in a proportion of the examined snails. The results indicated that *R. decollata* harbors different bacterial species and larval trematodes, as the first investigation in Iraq. The presence of these organisms indicates that *R. decollata* may play a role in the transmission of bacterial and parasitic infections, which may pose risks to public and animal health, and contribute to a better understanding of the epidemiological significance of terrestrial snails in the region.

Keywords: Land snails, *Rumina decollata,* bacteria, larval trematodes, Iraq

INTRODUCTION

Rumina decollata (Linnaeus, 1758), a terrestrial snail, belongs to the Mollusca: Pulmonata: Subulinidae, hermaphroditic, facultatively selfing, and extensively dispersed throughout the countries surrounds the Mediterranean Sea Northern Africa, Western Asia, and Southern Europe.[1,2] In the early 19th century, it was introduced globally.[3] *R. decollata* has moderately sized, fusiform, elongated shells that can reach a height of up to 4 cm, light grey to dark brown shells have weak growth lines on their exterior, and relatively deep sutures.[4] Previously, this species identified based on morphology and molecular recorded in Iraq for the 1st time by Bashê and Al-Qassab,[5] later by Ghulam[6] in the Middle Euphrates Region, Iraq.

In general, terrestrial gastropods are essential to ecosystems through soil formation, nitrogen cycling, and decomposition processes acts as prey for many predators, such as those mammals, birds, and insects[7] serves as a biological controls feed on mollusks, for example, the brown garden snail *Cornu aspersum*, even regarded as a plant pest.[8] Due to their feeding habits, the spread of infectious diseases, and their effects on certain economically important crops, land snails pose a significant threat. They proliferate quickly and have a detrimental impact on plants, particularly in areas that are suitable to their particular needs.[9-11]

Like other animals, terrestrial snail infected by various bacteria and parasites. The intestinal tract of terrestrial snails is perpetually exposed to bacteria from the external environment.[12] Furthermore, act as intermediate host of larval trematodes[13] have a complex life cycle[14] their definitive hosts are typically including birds and mammals.[15] The trematodes were identified as *Brachylecithum attenuatum*, which is now considered to belong to the genus *Lutztrema* and becomes *Lutztrema attenuatum*.[16] It has been documented from its definitive hosts, passerine birds globally, while it is larvae have never been observed in natural fields.[17]

R. decollata harbored the larval stage of the *Brachylaima* species when feeding mature eggs, obtained from adult flukes. Sporocysts develop only in *R. decollata*.[18] The recognition

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of hosts of *Brachylecithum* is still incomplete.[19] Taxonomic studies of the larval stages are less than comparable researches in their adult stage, due to the absence of species-specific morphological characteristics in the larval stages and difficulties in identifying the first and second intermediate hosts. Otherwise, the morphology of larval trematodes and their association with intermediate hosts can be of great assistance in streamlining trematode organization.[20]

In light of the above reasons, there are currently no reports on the isolation and identification of bacteria and trematodes that are associated with terrestrial snails in Iraq. This knowledge gap motivates the current work. The aim of this study was to isolate and identify bacteria species and larval trematodes infecting *R. decollate* snails obtained from Erbil province, Kurdistan region Iraq to improve understanding of their importance and potential effects on environmental and public health. In order to add them first time to the reported Iraqi.

MATERIALS AND METHODS

Sample Collection

R. decollata snails (n = 165) were collected from a moist garden in different locations (Mnara Park, Glkand P., Sami Abdulrahman P., Peshmerga P., Twraq Quarter, Brayati Q., Shawes Q., Kasnazan, Kawergosk) in Erbil province, Iraq, during September 2024 to August 2025. The sample sites are shown in Figure 1. The snails were placed in a container aeriated then transferred to the laboratory for identification and isolate of bacteria and trematodes.

Identification of Snails

Identification of the snail species depended on morphological traits such as shell height and the number of whorls. Measurements were obtained using a caliper with an accuracy of 0.1 mm. The shell description counts on the differences in shell diameter, height, and color. Compared and identified morphologically According to those resources.[21-24]

Isolation and Identification of Bacteria from *R. decollata*

Bacteria were isolated from *R. decollata* snails after surface (shell) sterilized by 70% ethanol to remove external contaminants.[25,26] The foot and digestive gland tissues were aseptically dissected, isolated bacteria by swabbing method, cultured in different medium such as Nutrient agar, Blood agar, and MacConkey agar prepared according to protocol.[27] After that incubated at 37°C for 24 h. then bacteria had growth in those medium and the assumed colonies of bacteria were identified manually by using methods Gram staining, biochemical tests such as (Indole test, TSI, Urease test, Citrate test), and automated the results were confirmed using a VITEK® 2 Compact system (Bio Merieux, Inc., France) according to protocol.[28,29]

Isolation and Identification of Larval Trematodes from *R. decollata* snails

Terrestrial snails were dissected using a crushing technique for isolation of parasites; the alimentary canal was cut into smaller segments and placed in petri dishes filled with the saline solution.[30,31] The sporocysts and the larvae of cercaria were observed under a dissecting microscope using a glass dropper put in a drop of water or Normal saline solution between glass slides and coverslips, stained (Hematoxylin and Eosin), cleared by xyline with fixed cercariae and unstained were properly examined via a compound light microscope for morphological characteristics.

RESULTS

A total of *(165) living samples of terrestrial snail species of R. decollata* were collected from various locations in Erbil City, Kurdistan Region, Iraq. During September 2024 to August 2025, average air temperature was arranged (2–40°C) and examined for isolation pathogens.

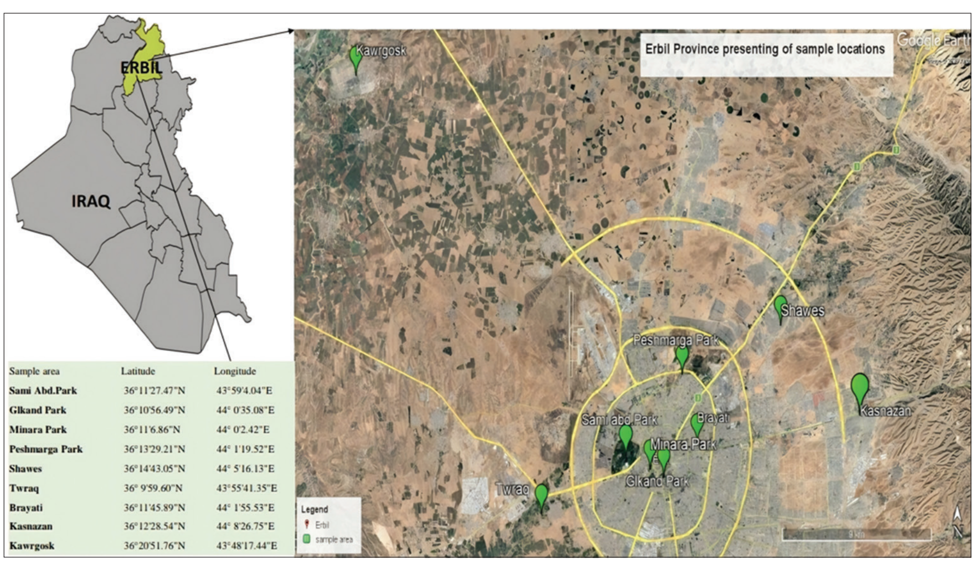

Figure 1: Map of Iraq, Erbil Province presenting of sample locations

Identification of *R. decollata* Snail

The shell morphology of the snail *R. decollata* reveals variations of shell length, which indicate variations in ages and the number of growth rings. The shell is long and cone-shaped with a decollate blunt end (only in adulthood), exhibiting a coloration ranging from tan to dark brown, shiny, and without bands; it comprises 3–7 whorls with a shell that measures 20–40 mm in length. The umbilicus is wide open. It is also observed that there is a simple left lip, while the right lip is almost not existing. The Body color was categorized as: light grey with a medio-dorsal black line, black, Black-grey. Foot color and body were scored as: pale grey, white-grey, beige, as shown in Figure 2.

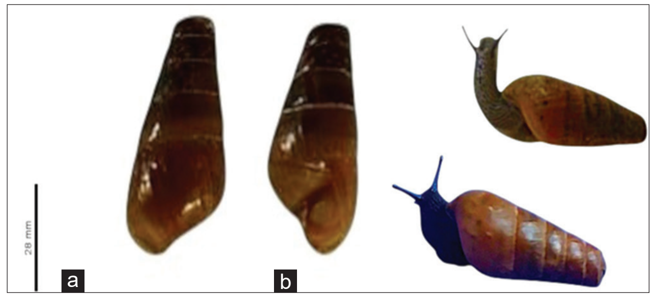

Figure 2: Shell of *Rumina decollata* snail; Left: (a: dorsal view; b: ventral view) (scale bar = 28 mm); Right: Living sample

Bacterial Identification that Isolated by *R. decollata*

Samples swabbing of *R. decollata* snails were cultured in different medium, the results showed that 102 samples snails had growth bacteria (62%) and 63 samples of snails had no growth (38%), as presented in Figure 3.

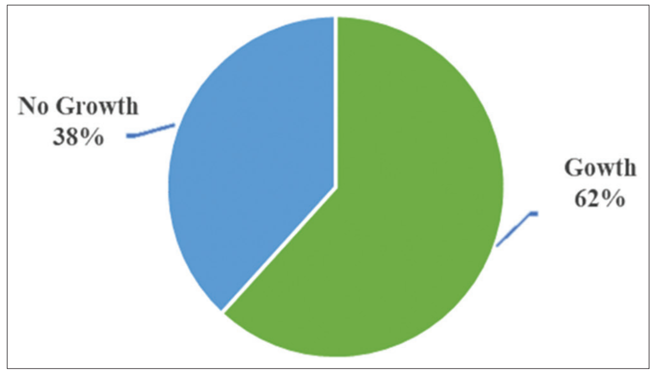

Figure 3: Prevalence rate of the occurrences of bacteria among examined snail *Rumina decollata*

Based on colony morphology, represented bacterial isolates were selected for analysis. The colonies presented variable morphological characteristics across different culture media. On MacConkey agar, lactose-fermenting colonies appeared pink, whereas non-lactose-fermenting colonies remained colorless. Microscopic examination of Gram-stained bacterial cells demonstrated diverse morphologies among isolates, as shown in Figure 4, characterization based on biochemical analysis shows a predominance of Gram-negative in the isolates, and automated identification with VITEK II subsequently employed to confirm the bacterial identities. As appeared in Figure 5. Multiple bacterial species exhibiting distinct colony morphologies were isolated from *R. decollata*. The recovered isolates were assigned to four bacterial families comprising Enterobacteriaceae, Lysobacteraceae, Pseudomonadaceae, and Moraxellaceae.

The most frequently identified species included *Klebsiella pneumoniae* (20%), whereas the other identified species included *Citrobacter koseri* (*diversus*) (15%), *Escherichia coli* (10%), *Enterobacter aerogenes* (10%), *Enterobacter cloacae* (10%), *Acinetobacter baumannii* (10%), *Citrobacter freundii* (5%), *Stenotrophomonas maltophilia* (5%), *Pseudomonas mendocina* (5%), *Pantoea agglomerans* (*Enterobacter agglomerans*) (5%), and *Acinetobacter calcoaceticus* (5%). being the most common isolates are illustrated in Figure 6.

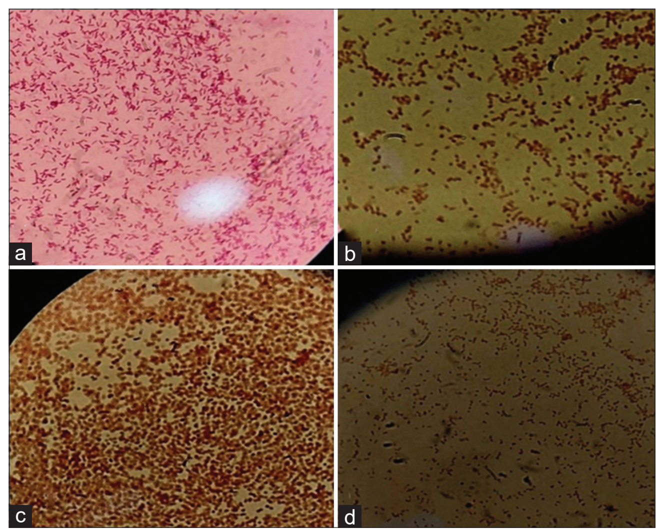

Figure 4: Bacterial Gram staining under 1000×, (a) *Klebsiella pneumoniae*; (b) *Pseudomonas mendocina*; (c) *Acinetobacter baumannii*; (d) *Stenotrophomonas maltophilia*

Identification of Larval Trematodes Isolated by *R. decollata*

Terrestrial Snail *R. decollata* were collected and examined for larval Trematodes. Among the examined snails, eight individuals of *R. decollata* were infected with larval Trematodes (sporocysts containing cercariae) of *L. attenuatum* (*B. attenuatum*) [Figure 7]. These larvae particularly appeared to be a member of the family Dicrocoeliidae, the daughter sporocysts exhibited an unbranched tubular character, and each cercaria featured an oral stylet along with a simple, unforked tail.

The cercaria of *B. attenuatum* belongs to the xiphidiocercaria group, specifically a type often described as brevicaudate or microcercous, tails lack fin folds or spines. They have a stylet in the oral sucker and a small, knob-like structure.

DISCUSSION

In this investigation, various bacterial species obtained from *R. decollata* through standard isolation procedures. The prevalence was fluctuated; these isolates constitute a

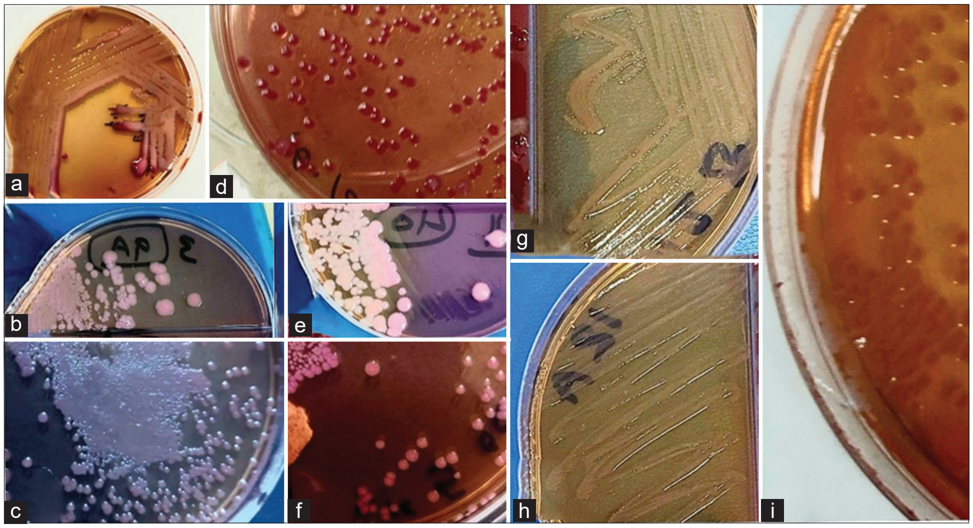

Figure 5: Bacterial growth on MacConkey agar, (a) *Klebsiella pneumoniae;* (b) *Enterobacter aerogenes*; (c) *Citrobacter koseri* (*Citrobacter diversus*); (d) *Escherichia coli*; (e) *Enterobacter cloacae*; (f) *Citrobacter freundii*; (g) *Stenotrophomonas maltophilia*; (h) *Pseudomonas mendocina*; (i) *Acinetobacter baumannii*

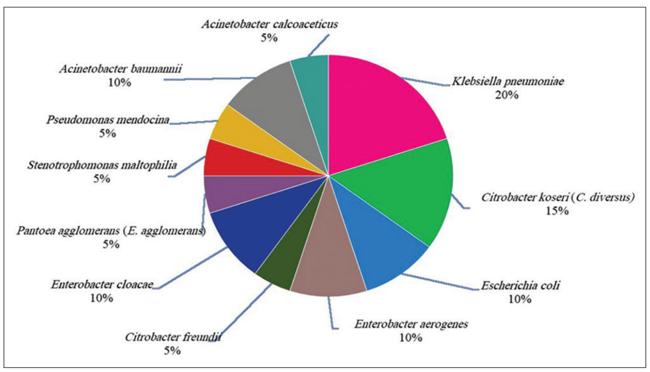

Figure 6: Identification of growth bacteria in culture media isolated from *Rumina decollata*

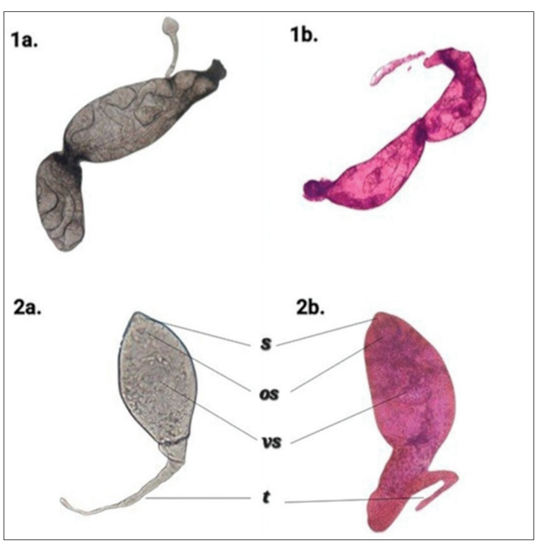

Figure 7: *Lutztrema attenuatum*, 1: Sporocyst under 100×; 2: Cercaria under 400× (a: Unstained; b: Stained), s: Stylet, os: Oral sucker, vs: Ventral sucker, t: Tail

heterogeneous assemblage of ambient and opportunistic bacteria often located in soil and within snail gut. Prior study showed that one important area where a snail's physiology and ecology interact is their gut. Microorganisms from its environment can permanently invade it, resulting in beneficial or detrimental effects.[12]

The present research highlights the most frequent *K. pneumoniae* among bacterial isolates, conversely minimum prevalence rates (5%) were observed in *C. freundii*, *S. maltophilia*, *P. mendocina*, *P. agglomerans* (*E. agglomerans*), and *A. calcoaceticus*. Consistent with prior evidence, these isolation rates are attributed to snail often contacts moist soil and decomposing vegetation, it easily picks up *Klebsiella* from its surroundings.[32] The suggested actions for snail *R. decollata* in garden areas can affect other organisms. Infected snails may carry and transmit pathogenic bacteria to plants, soil organisms, and other animals in the ecosystem, according to Caullan *et al*.[33] illustrated that infected snails with *Pantoea* and *Klebsiella*, which catalyze disease development, moreover suggests these bacterial species deplatt *Lactobacillus* in *Cornu aspersum*. In humans, *K. pneumoniae* serves as a clinically significant pathogen capable of causing severe and potentially life-threatening pneumonitis, a broad spectrum of hospital-acquired infections, encompassing meningitis, septicemia, thrombophlebitis, osteomyelitis, and urinary tract infections.[34]

E. coli was found in *R. decollata*, and that's consistent with previous findings from research on other terrestrial snails. Hence, many studies have reported that Snails deposit feces and mucus on garden plants. Bacterial infections from snail excrement in gardening could be directly ingested by humans.[35] Avery *et al*.[36] found that snails can retain both pathogenic and lab stains of *E. coli* for 30 days after a single exposure, despite being cold-blooded. In contrast, *E. coli* from livestock persists on grass and infects warm-blooded animals. Thus, snail feces may act as a key environmental reservoir for *E. coli* transmission to humans and animals.

Moreover, various bacteria recovered in this study demonstrate distinct roles in the environment and within the bodies of *R. decollata*, Based on previous studies have reported several snail species and the isolation of diverse bacteria, including *Citrobacter, Enterobacter, Pseudomonas, Acinetobacter*, *Kluyvera*, *Pantoea* and *Escherichia* look like to be the fundamental microbiome of terrestrial snails, because at least four distinct snail species from the evaluated research have been shown to have them. However, the identical species within the core microbiota of freshwater and marine snail observations were also detected in land snails.[7] *Citrobacter, Klebsiella, Enterobacter, Staphylococcus, Streptococcus,* and *Vibrio* were earlier noted in the snail gut as facultative anaerobic bacteria. A small portion of cultivated snail strains of Enterobacteriaceae and Enterococci resides in animal intestines, despite their usual association with enteric environments.[37] This variation likely reflects differences among snail species and their regional distributions.

Furthermore, two *Acinetobacter* species, specifically *A. baumannii* and *A. calcoaceticus,* were identified in this study, demonstrated bacterial diversity associated with *R. decollata*. In accordance with previous reports, *Acinetobacter* predominates in the microbiomes of snails.[38] Earlier studies have documented the isolation of cellulitic Acinetobacter from terrestrial snails, which is associated with their plant-based diet.[39] *A. baumannii* is a ubiquitous opportunistic pathogen that frequently colonizing the human respiratory tract, skin, and genitourinary system, ability to form robust biofilms facilitates transmission and enhances environmental persistence.[40]

The species of the bacteria in the snail's gut are classified into two taxa; γ-Proteobacteria (*Buttiauxella*, *Citrobacter*, *Enterobacter*, *Obesumbacterium*, *Kluyvera*, *Raoultella*) and Firmicutes (*Lactococcus*, *Enterococcus*, *Clostridium*). Typically derived from enteric or phyllosphere habitats, facilitating snail cultivation with soil and plants.[41] Alterations in the microbial flora of snails could adversely affect its feeding behavior, perhaps leading to death.[42] Few studies have examined snail microbiomes; the bacterial microbiota identified in the gastrointestinal tract (GT) of snails may play a significant role in digestion process. The functionally specialized areas of the GT may signify various microenvironments and could consist of atypical bacterial populations.[42] Maintenance of a balanced microbial community and averting dysbiosis (adverse health consequences resulting from a disrupted microbiome) is essential for the host's health. Several factors can affect the compositions of animal gut microbiomes, consisting of seasonality, pathogens, host diet, and disease.[26] On entering the GT, these bacteria may proliferate, be lysed by intestinal enzymes, or be expelled.[12]

The current study Isolated larval trematodes of *L. attenuatum* from *R. decollata* for the first time in Iraq. This species can infect trematodes as the first intermediate host and ingested by other hosts, trematode and nematode infections have been previously reported for *R. decollata*, including sporocysts belonging to the family Dicrocoeliidae parasites from mammals and birds in life cycles complex. *R. decollata* has not been reported as an intermediate host for Dicrocoeliidae, yet this report is a remarkable nuance in its parasitological role.[43] Previously documented of Dicrocoeliidae sporocysts and cercariae of *L. attenuatum* were detected from the land snail *Allopeas satsumense* (Pilsbry, 1906) in Japan.[44] Characterizing parasitic taxa, specifically trematodes, provides critical insights into the parasitic biodiversity of Iraq. These findings broaden the known biogeographical range of the dicrocoeliidae trematode in the Middle East and add data to the helminth fauna of Iraqi terrestrial gastropods.[45] Land snails shell-encapsulate trematode cercariae by hemolytic aggregation and melanization this effective physiological response transmit parasites preventing infection. This encapsulation mechanism reflects the evolutionary arms race between digenetic trematodes and their molluscan first intermediate hosts, wherein snails deploy both cellular and humoral immune responses to inhibit parasite establishment.[46] The presence of *L. attenuatum* confirmed significant physiological modifications in host snail *R. decollata* and impact other organisms.

This study revealed that cercariae from *R. decollata* had common morphological characters like a minute stylet on an oral sucker and a simple, unforked tail, as shown in Figure 7. These results can be confirmed by forms of the cercarial tail. Larval trematodes tail belongs to the xiphidiocercaria type, and these long tails are features in cercariae of *Brachylecithum, Lutztrema,* and *Dicrocoelium.*[15] The status and classification of numerous Dicrocoeliidae species remain uncertain. Several of the current descriptions are inadequate, due to the important identification properties are not clearly visible, and due to molecular analyses have not confirmed the specificity about the morphological identification characters, except in limited economically crucial species. In Central Europe, Avian Dicrocoeliidae were investigated through three recently published studies.[47]

Knowing the species and isolated trematodes from terrestrial snails such as *R. decollata* is important for the surroundings and public health. Snails are likely to have an increased risk of infection by a greater variety of trematode species in situations where vertebrate hosts are more prevalent and diversified.[48] Identification of parasites infecting snails and their sources or reservoirs of infection under agricultural situations is essential to mitigate the harmful effects these representatives and decrease the possible risks for human health associated with particular parasites in snails.[49]

Finally, depend on documented recorded, *R. decollata* acts as source of different bacterial species and larval trematodes may lead to effect on agriculture fields and human healthy. Hence, research focuses on snails as agricultural pests due to their resilience, rapid reproduction, and diverse feeding habits, which disrupt ecosystems. Without biological, chemical, and mechanical management, they spread and outcompete other species.[50] *R. decollata* was expected to become a horticultural pest worldwide due to its generalist habits, self-fertilization, and high reproduction rates.[11] Children in particular susceptible to direct contacts with snails when playing in residential zones and Public parks, where they may touch the snails or their excretions, such as mucus and feces that transmit human pathogens.[51]

CONCLUSIONS

Our investigation demonstrates that *R. decollata* acts as hosts for pathogens (Bacteria and Parasites), particularly where snails occur near humans, livestock, and wildlife. Detection a wide variety of bacterial including environmental and opportunistic taxa species and larval trematodes, indicates ongoing transmission and possible risks to animal health, such as Birds, Mammals with indirect implications for public health.

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