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A COMPREHENSIVE STUDY ON THE INFECTIVITY *OF GALBA TRUNCATULA* (O. F. MÜLLER, 1774), AN INTERMEDIATE HOST OF *FASCIOLA* SP. REPORTED IN DİYARBAKIR HOSPITALS

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ABSTRACT. This study aims to comprehensively investigate the trematode infection in *Galba truncatula* (O. F. Müller, 1774) a widely distributed intermediate host in the Dicle River, following the previously reporting of Fascioliasis cases in Diyarbakır hospitals. While previous literature has documented the presence of *F. hepatica* and *F. gigantica* in individuals treated in Diyarbakır hospitals, the origin of the trematodes remains unspecified. In this study, *G. truncatula* samples were collected from three densely populated areas along the Dicle River, specifically Hevsel Gardens, and subjected to histopathological examination to determine the parasite's infectivity. The analysis revealed that the snails were not infected with trematodes; however, the presence of the host and its wide dispersion in the Dicle River pose a significant risk of disease in the future. Hevsel Gardens, a location where sewage and wastewater from the northern suburbs of Diyarbakır converge with the river, is extensively used for activities that involve direct contact with *G. truncatula*. These activities include vegetable cultivation, fishing, farming, husbandry, and swimming, and pose an escalating risk of potential contagion. The study acknowledges the undetermined source of the parasite but highlights its preliminary nature, emphasizing the urgency of proactive measures. The findings will enhance our understanding of the health risks linked to *G. truncatula* and underscore the significance of implementing effective control measures in a timely manner.

1. INTRODUCTION

Galba truncatula (O. F. Müller, 1774), an aquatic pulmonate gastropod, is believed to have originated in Europe but has since expanded its distribution to various regions worldwide and not typically considered an exotic or invasive species. It can now be found in all European countries, including numerous Mediterranean islands such as Corsica, Malta, the Azores, Madeira, the Faroe Islands, the Balearic Islands, and the Canary Islands [1]. Furthermore, recent discoveries have identified its presence in North and South America, Africa, and Asia, including countries such as Russia, Iran, Pakistan, and India [1]. While *G. truncatula* is predominantly found in northern Africa, encompassing Morocco,

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Algeria, Tunisia, and Egypt, it has also been documented in South Africa, Ethiopia, Kenya, and Tanzania [1–3]. *Notably, comprehensive distribution maps detailing the exact range of G. truncatula across the globe are currently unavailable* [1].

G. truncatula is characterized by its conical, slightly turreted shell with swollen whorls and deep sutures. The shell, thin and mid-brown in colour, consists of four distinct whorls with prominent sutures (Fig. 2). Typically, the height of the shell ranges between 6 and 11 mm, while the width falls within 3 to 6 mm. This amphibious snail species inhabits areas near small streams, rivers, and various wetlands, exhibiting a preference for still or slow-moving water. Interestingly, it demonstrates adaptability by venturing onto land, frequently occupying damp field margins, wet hollows, and even dried-up puddles. In terms of diet, *G. truncatula* feeds on algae as well as fresh or decomposed plant matter. Its ability to crawl considerable distances from open water contributes to its widespread distribution and presence in diverse environments.

Trematodes, commonly referred to as flukes, are parasitic organisms that have a distinct life cycle requiring multiple hosts. They are obligate internal parasites, meaning they rely on other organisms for their survival and reproduction. The life cycle of trematodes involves an intermediate host, often a snail, where asexual reproduction takes place, and a definitive host, typically a vertebrate, where sexual reproduction occurs. These parasites have the ability to cause diseases in a wide range of vertebrates, including mammals, birds, amphibians, reptiles, and fish. The impact of trematode infections on various vertebrate classes highlights the significant health risks associated with these parasites [4]. Within the family Lymnaeidae, freshwater snails have a critical role as obligatory hosts for trematodes. In Central and South America, species such as *Lymnaea viator*, *L. neotropica*, *L. cubensis*, and *Pseudosuccinea columella* fulfill this role. In Australia, *L. tomentosa* is involved in the transmission of trematodes, while in the United States, *Fossaria modicella* and *Stagnicola bulimoides* are relevant. However, it is *G. truncatula* that serves as the primary vector responsible for transmitting trematodes to humans across Europe, Asia, Africa, and South America [4–6].

G. truncatula plays a crucial role as the intermediate host for several trematode species, including *Fasciola hepatica, F. gigantica, Fascioloides magna, Haplometra cylindracea*, *Plagiorchis spp.,* and *Paramphistomum daubneyi.* These parasites have been extensively studied and documented in researches [1,7–9]. These studies have provided valuable insights into the transmission dynamics and biology of these trematodes, furthering our understanding of the complex interactions between *G. truncatula* and these parasite species.

Fascioliasis, a parasitic trematode infection caused *by F. hepatica* and *F. gigantica,* typically occurs when individuals unintentionally ingest the parasite. The most prevalent mode of transmission involves the consumption of contaminated water or the ingestion of tainted vegetables, particularly watercress or meats. Moreover, individuals can acquire the infection by consuming foods that have been washed or irrigated with contaminated water [10–12]. In rare circumstances, people may become infected by consuming undercooked sheep or goat liver containing immature forms of the parasite. It is important to note that direct person-to-person transmission of *Fasciola* does not occur. For the parasite to infect another individual, the eggs excreted in the feces of infected individuals (and animals) must develop within specific types of freshwater snails under favorable conditions [10–12].

In an aquatic or highly moist environment, the eggs of *Fasciola* undergo a three-week incubation period at 15°C and hatch when temperature conditions become favorable, releasing miracidia [13]. These miracidia actively swim for several hours, seeking out a suitable freshwater snail host for penetration. While many snails may be receptive, only a select few can support the complete larval development of the parasite. *G. truncatula* fulfills this crucial role, as its presence is vital to the parasite's life cycle [7,9,10,13]. Upon penetration of the snail, the majority of miracidia enter the snail's tissue near the pulmonary cavity, shedding their epithelial plates and cilia in the process. Within 12 hours, a miracidium undergoes metamorphosis into a mother sporocyst. The mother sporocyst develops and produces the first generation of eight daughter rediae, subsequently rupturing the sporocyst's wall. These initial rediae can give rise to a second generation of rediae, both of which generate a significant number of cercariae. The cercariae exit the rediae through the birth pore and must mature within the snail's tissues before emerging. Typically, the cercariae emerge from their intermediate snail host, *G. truncatula*, during autumn, often triggered by bright light [14]. After emerging, the cercariae swim to the water's surface, shed their tails, and encyst themselves, transforming into metacercariae. These metacercariae attach to vegetation near the water's edge [14].

The findings of this study will contribute to a better understanding of the epidemiology and transmission patterns of *F. hepatica* and *F. gigantica* in the region. By pinpointing the prevalence of these trematodes in *G. truncatula*, it will be possible to assess the potential risks of human infections and implement appropriate control measures, if necessary. Additionally, the research outcomes will help in devising strategies for the effective management and prevention of trematode infections associated with *G. truncatula* in the Dicle River area of Diyarbakır.

2. MATERIALS AND METHODS

Sample collection

The study involved the collection of *G. truncatula* samples from three distinct locations along the Dicle River, each with specific coordinates for precise identification. Location 1 was situated under the Dicle University Bridge (37°55'15.5"N, 40°14'56.3"E), while location 2 was in the center of Hevsel Garden (37°53'34.4"N, 40°15'06.5"E). Lastly, location 3 was near the Ongözlü Bridge (37°53'22.9"N, 40°13'47.8"E). The snail sampling was conducted during autumn, a critical period characterized by the increased reproduction and multiplication of trematodes. To ensure personal safety, protective gloves were used during the collection process, and the snails were carefully placed in plastic containers filled with river water. Approximately 300 snail samples were collected throughout the designated search period. To preserve the snail samples, they were immediately transferred to universal tubes containing moist tissue paper and kept cool. Within a maximum of 5 hours, the samples were transported back to the laboratory. Once in the laboratory, the snails were stored in a deep freeze at a temperature of -20°C, ensuring their preservation for subsequent analysis. Within 12 hours after collection, the snails underwent morphological recognition, employing specific criteria described in earlier references [15–18]. These criteria included shell shape and measurements, the number of whorls, aperture shape and position, and width. The assistance of malacologist Rıdvan Şeşen from the Department of Biology at Dicle University facilitated accurate identification of the samples. To document the shells of the collected samples, high-resolution digital microscope cameras (Jiusion wifi USB Digital Microscope 50 to 1000×) were employed, enabling detailed and clear photographs (Fig. 2). For parasitic analysis, 40 mature snails were selected from each of the three locations, resulting in a total of 120 snails being utilized for this specific aspect of the study.

Histopathological preparation

After a period of three days, the collected snails were transported to the laboratory at Gazi Yaşargil Training and Research Hospital under controlled temperature conditions of 21°C. During the necropsy procedure, the shells of the snails were delicately removed. Subsequently, the snail tissues underwent a dehydration process and were embedded in paraffin at a temperature of 56°C. To ensure proper histopathological examination, the whole tissues were immersed in a neutral buffered 10% formalin solution and sent to the pathology laboratory. The tissues were fixed in the formalin solution for a duration of 24 hours. Following fixation, the tissues were embedded in paraffin blocks using a Leica ASP® 300 automatic vacuum tissue tracking device manufactured by Leica Microsystems in Germany. Sections of the prepared paraffin blocks, with a thickness of 4 µm, were obtained using a Leica RM® 2135 rotary microtome device, also from Leica Microsystems. These sections were then mounted on slides and subjected to staining using Hematoxylin-Eosin (H&E) stains, enabling normal histological evaluation. The prepared slides were carefully examined under an Olympus BX53F light microscope (Olympus, Tokyo, Japan). To document the findings, photographs were taken using an Olympus E-330 camera, ensuring detailed visual representation of the histopathological specimens.

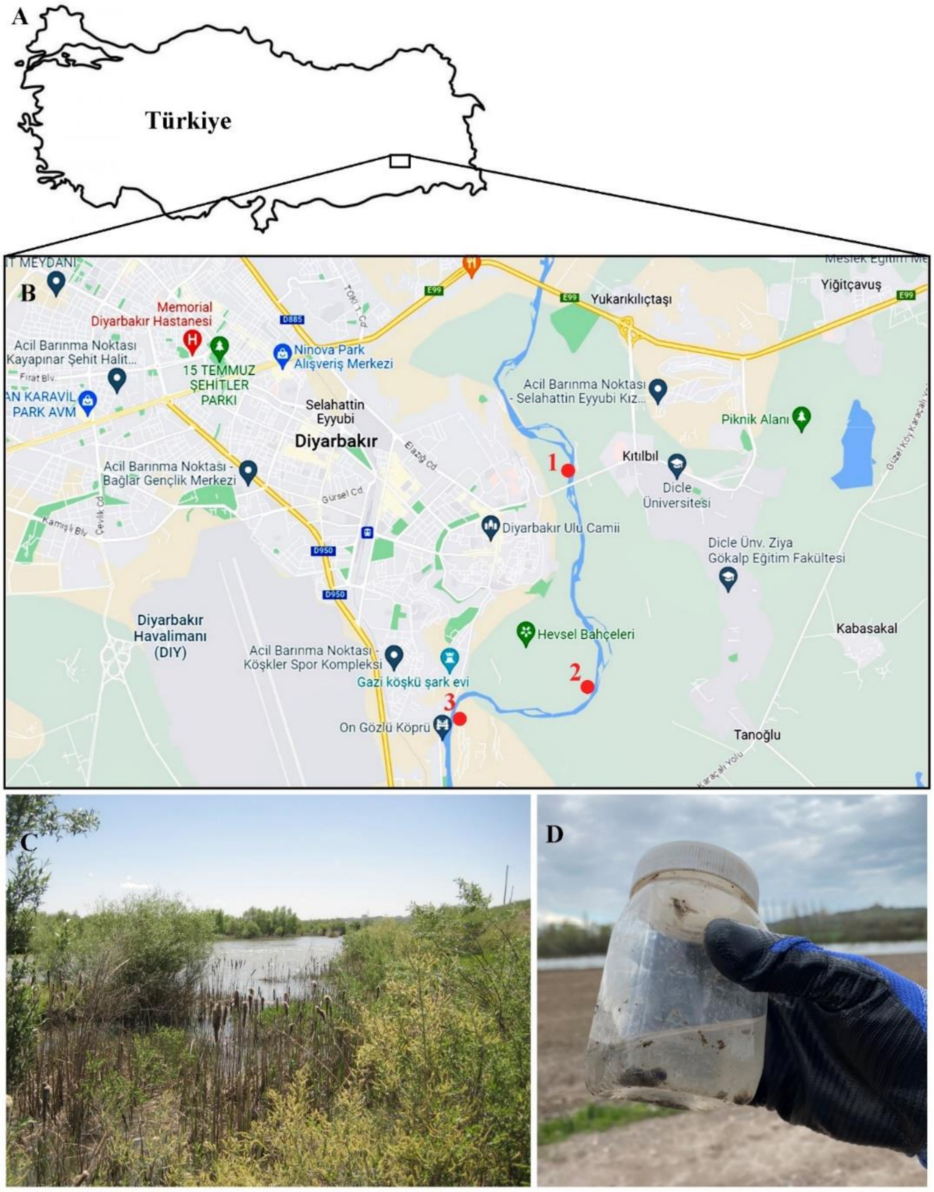

FIGURE 1. A. The map of Türkiye, B. The map of Diyarbakır, and three collection areas of *Galba truncatula*, C. The bank of the Dicle River, D. The samples in a plastic container.

FIGURE 2. The morphology of *Galba truncatula*, focusing on key features such as the shape of the shell, the number of whorls, the aperture, apex, body whorl, umbilicus, and sutures. The image includes a black bar measuring 1 mm, serving as a reference for scale.

3. RESULTS AND DISCUSSION

In terms of infection dynamics, once the miracidium enters a snail host, it undergoes a transformation into a sporocyst. Depending on the outcome, the sporocyst either survives the initial week of infection and gives rise to first-generation rediae (snails with active infections), or it perishes within the first week (snails with abortive infections) [19]. While the presence of living parthenitae in dissected snails easily confirms active infections, identifying abortive infections requires histological investigation, which detects residual parasites and identifies any tissue lesions within the snail's organs. Histological investigation also enables differentiation between snails with abortive infections and uninfected snails that have not experienced miracidia penetration [19]. In general, the presence of rediae serves as an indicator of snail infection, and their observation in histological sections helps assess the extent of the infection. *However, in the current study's histopathological sections of G. truncatula, no cercariae, sporocyst, or notably rediae of F. hepatica and F. gigantica were observed* (Fig. 3). Previous investigations of the *G. truncatula-Fasciola hepatica* model have documented histological alterations in snails with active infections. Notably, the albumen gland, digestive gland, gonads, and kidney of these snails displayed epithelial necrosis followed by regeneration [19,20]. However, in the present study, no signs of epithelial necrosis or regeneration were observed in the albumen gland, digestive gland, gonads, or kidney of *G. truncatula*, indicating the absence of infection with *F. hepatica or F. gigantica* (Fig. 3).

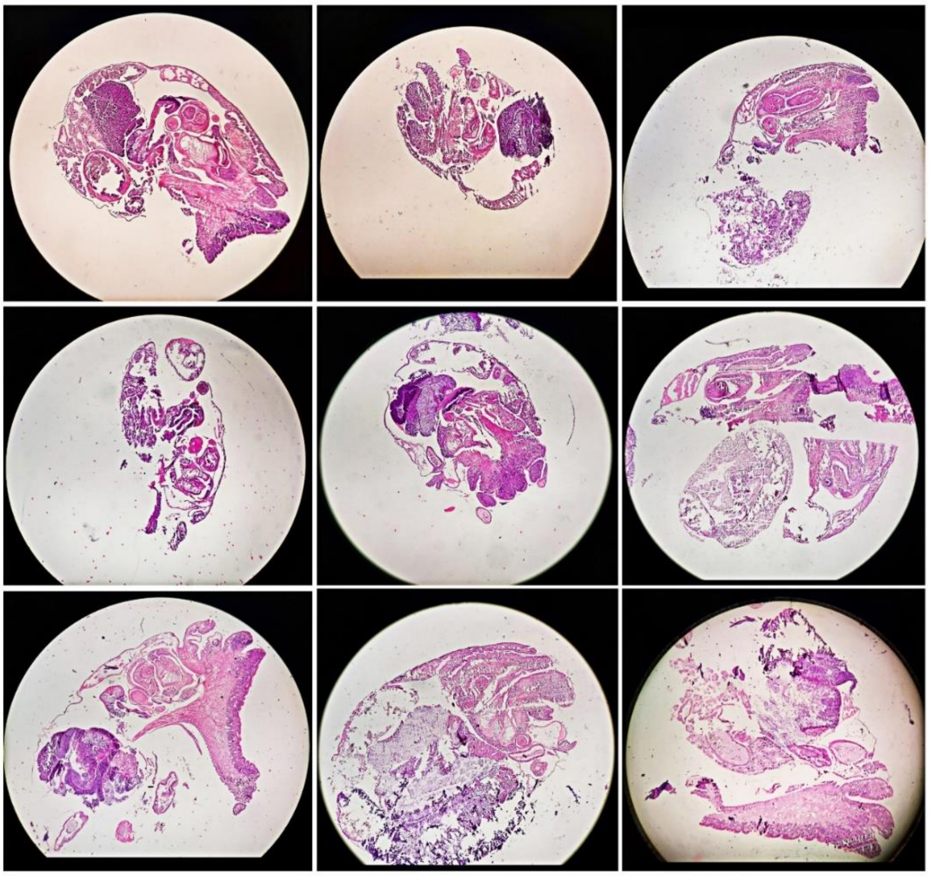

FIGURE 3. Microscopic images of different histological sections of *G. truncatula*. Notably, no evidence of trematodes was detected in any of the examined sections.

No recorded literary works have specifically explored *F. hepatica* and *F. gigantica* infestations in *G. truncatula* within the context of the Dicle River. Nevertheless, some documented cases of Fascioliasis treated in hospitals in Diyarbakır have been reported in the literature. In a published paper, a 44-year-old female patient presented to the Department of Gastroenterology at Dicle University Medical Faculty with symptoms of abdominal pain, nausea, vomiting, anorexia, and weight loss. Following comprehensive diagnostic assessments involving laboratory tests and imaging techniques, the patient was initially admitted to the hospital with a provisional diagnosis of cholangitis-cellular carcinoma. However, after a meticulous examination, the patient was ultimately diagnosed with *F. hepatica* through the utilization of Endoscopic Retrograde Cholangiopancreatography (ERCP) [21]. In another study conducted at the Department of Gastroenterology, Dicle University Faculty of Medicine, a 46-

year-old woman sought medical attention for right upper quadrant abdominal pain and jaundice. Upon evaluation, the patient was diagnosed with *F. gigantica* [22]. Additionally, a separate article reported the examination and treatment of several patients with Fascioliasis who presented at the Surgery Clinic of Dicle Medical Faculty [23]. A study focusing on *F. hepatica* in small ruminants in the vicinity of Diyarbakır, particularly Siirt, revealed the presence of *F. hepatica* in six out of 3,193 cattle and one out of 3,659 small ruminants [24]. Moreover, reports of Fascioliasis in small ruminants have emerged from neighbouring regions close to Diyarbakır, including Van, Tatvan, Elazig, Malatya, and Hakkari [24–26]. However, there is a lack of available information in the literature regarding the precise origins of these parasites. It is plausible that patients acquired the trematodes through the consumption of undercooked meat, such as from goats, sheep, or cattle sourced from various locations. Fascioliasis can be transmitted from large livestock, such as cattle or sheep, to humans through a specific transmission pathway. The life cycle begins with these animals grazing on pastures contaminated with Fasciola metacercariae, the infectious stage of the parasite. Upon ingestion, the metacercariae develop into adult parasites within the host's liver. Humans can become infected when they consume raw or undercooked animal products derived from these infected animals or when they ingest water or plants contaminated with metacercariae. This transmission pathway highlights the zoonotic potential of Fascioliasis and the importance of proper food safety practices and the control of contaminated water sources in preventing human infections. Another potential source could be the ingestion of contaminated vegetables, particularly watercress, or the consumption of water containing parasites in their rural areas. It is worth noting that the hospitals in Diyarbakır attract patients from neighbouring provinces and districts due to their comprehensive and advanced medical facilities. This raises the possibility that the parasites may have originated from outside of Diyarbakır. The patients could have potentially come into contact with *G. truncatula*, which typically inhabits lakes, rivers, canals, and stagnant water ponds in areas surrounding Diyarbakır. According to the literature, it is known that the parasite can persist within the human body for up to 13.5 years [27]. Hence, it is also possible that some patients may have been harboring the parasite for an extended period. Another consideration is that the cited literature was published in 2014, suggesting that patients might have been infected prior to 2014, and the infection may have resolved in the intervening time. Considering these various possibilities, it becomes exceedingly challenging to identify the precise source of the parasite. However, by obtaining information on the residences of patients with Fascioliasis who seek treatment in hospitals in Diyarbakır, it may be possible to gain further insights into the potential sources of the infection.

4. CONCLUSIONS

In summary, the significance of *G. truncatula* becomes evident as it serves as an essential intermediate host for Fasciola species in parasitology research. Moreover, its presence and wide distribution in the Hevsel Gardens along the Dicle River, a habitat it has inhabited since 1992 [28], underscore the potential risks associated with its presence in this ecosystem. This highlights the importance of closely monitoring the implications of its presence for both parasitological studies and the conservation of freshwater ecosystems. The discharge of sewage waters from suburban areas into the river, coupled with the lack of infrastructure, creates an environment conducive to the occurrence of Fascioliasis. Limited data on the prevalence of the disease further accentuate the need for immediate action. To safeguard the local population from these diseases, it is crucial to detect the presence of *F. hepatica* and *F. gigantica* in the river and *G. truncatula* in future studies before it becomes too late. Additionally, addressing the issue of wastewater discharge from neighborhoods without a wastewater network is essential. Moreover, further investigation of the aquatic environments in the districts where treated patients reside would provide valuable insights into the potential as an intermediate host for *G. truncatula*. Despite the challenges posed by patient rights and privacy, it is crucial to gather more information to better understand and control the spread of this species. Overall, this study serves as a preliminary call to action, emphasizing the importance of proactive measures to mitigate the risks associated with the presence of *G. truncatula* and protect the well-being of the local community.

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Author Contribution Statements İE contributed extensively to the manuscript, including the approval of the final version, critical literature review and interpretation, material collection and preparation, manuscript critical review, preparation and writing. AY contributed specifically to the histopathological analysis and section investigations.

Declaration of Competing Interests The authors declare no conflict of interest.

Ethics Committee Approval As this article does not involve any studies with human or vertebrate animal subjects, it was not necessary to obtain approval from an ethics committee.

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