Capillaria hepatica in Rattus Spp. Captured in Sanandaj and Orally uninfected Balb/C Mice With Embryonated Eggs

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Abstract

Introduction: *Calodium hepaticum* (syn. *Capillaria hepatica*) is a nematode with worldwide distribution among rodents (*Rattus rattus*, *Rattus norvegicus*) which can also infect human beings through ingestion of embryonated eggs and cause the zoonotic disease of capillariasis philippinensis. This study aimed to determine the *C. hepatica* infection in *Rattus* spp. in Sanandaj, Kurdistan province of Iran, as well as orally infecting of Balb/c mice with embryonated eggs.

Methods: Area of study was the city of Sanandaj located in the west of Iran, wherein 4 *R. norvegicus*, 6 *R. rattus* and 10 *Mus musculus* were captured using live traps in a period of more than 2 years. Livers were examined for *C. hepatica* infection, sectioned, and then stained with hematoxylin and eosin. Embryonated eggs were used in order to experimentally infect 3 Balb/c mice orally.

Results: *Capillaria hepatica* was detected in 5 out of 20 livers (25%); rates of infection were 50% (2/4) for *R. norvegicus*, 50% (3/6) for *R. rattus*, and 0% (0/10) for *M. musculus*. Cross sections of the livers showed active infections as eggs were deposited beside the worms, and they were also observed inside the female worms. After performing the autopsy on 3 mice that had been infected experimentally, there was no sign of infection based on gross and microscopic examinations.

Conclusion: This study was the first report on *C. hepatica* infection in *Rattus* spp. in Sanandaj. The main reservoir hosts of *C. hepatica* infection in different areas of Sanandaj were *R. rattus* and *R. norvegicus*. On the other hand, with regard to limited sample size of study, no significant role could be considered for either infection or transmission of *C. hepatica* for *M. musculus* in these areas.

Keywords: Parasitic infection, *Calodium hepaticum*, *Capillaria hepatica*, Liver, Rodent

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Introduction

Capillaria hepatica (syn. *Calodium hepaticum*) is a nematode, first described by Bancroft in 1893. The parasite belongs to the order of *Trichinellida* and *Capillariidae*.¹ It is distributed worldwide among rodents, and also has been found in more than 140 mammalian species such as human beings, which causes the zoonotic disease of capillariasis.² The size of an adult parasite is ranged from 30 to 50 mm in length in males and females.¹ The eggs are bi-operculate-structured with a tray-like shape, and have a pitted shell, and the apical plugs do not protrude.³ Eggs are embryonated within 5

to 7 weeks outside the host and transmitted directly through being ingested by the soil or animal carcasses without the need for an intermediate host. After ingestion of infective eggs, L1 larvae migrate to the cecum, penetrate into the intestinal walls, enter the bloodstream, and finally reach the hepatic parenchyma, where they turn from L2 into L4 larvae, and then into adult worms in about 1 month. The mature adult worms mate and release the cluster of fertilized eggs around the female releases. The female dies after a period of approximately 30 days after laying eggs, and immature fertilized eggs remain viable up to 120 days

in host's liver.⁴ In 1924, the first human infection was reported by McArthur, and after that, this infection was reported occasionally from different parts of the world.⁵ Two infection types caused by *C. hepatica* are: spurious infection when host ingests unembryonated eggs, and true hepatic infection following the ingestion of embryonated eggs.⁶ Site of true infection is liver parenchyma, where in case of human infection, symptoms such as prolonged fever, splenomegaly, acute liver inflammation, peritonitis, abdominal pain, ascites and eosinophilia may occur, and occasionally cause severe and fatal infections.^{7,8}

Rodents are the largest mammalian group on the earth.⁹ Synanthropic rodents that live in close contact with human habitats in urban areas worldwide are house mouse (*M. musculus*), black rat (*R. rattus*), and brown rat (*R. norvegicus*).¹⁰ One of the critical public health problems to human populations are *Rattus* spp. which harbor endoparasite nematodes such as *Capillaria hepatica*, *Aspiculuris tetraptera*, *Syphacia obvelata*, *Trichinella* spp., *Strongyloides muris*, *Trichosomoides crassicauda*; trematodes such as *Schistosoma* spp.; cestodes such as *Hymenolepis nana*, *Hymenolepis diminuta*, *Taenia taeniaeformis*; acanthocephalans such as *Moniliformis moniliformis*; protozoa such as *Cryptosporidium* spp., *Babesia* spp., *Trypanosoma* spp., *Leishmania* spp., *Trichomonas muris*, *Giardia* spp, *Sarcocystis* spp.,¹¹⁻¹⁴ and many other parasites, bacteria and viruses that most of them could cause zoonotic diseases in humans.¹⁵

Rodents, especially the genus *Rattus*, are considered to be the main reservoir and primary host of *C. hepatica*¹⁵ in which prevalence rate of infection can reach to 100% in some areas, and this infection has been reported from many parts of the world¹⁶. Active life cycle of the parasite in different geographical locations can be a potential risk for the public health¹⁷; so this study aimed to determine the *C. hepatica* infection in *Rattus* spp. in Sanandaj, Kurdistan province of Iran, and investigate the sensitivity of laboratory mice to the maintenance of the parasite in vivo with embryonated eggs orally.

Methods

Area of Study

Sanandaj is the capital of Kurdistan province located at 35° 18' 52" N, 46° 59' 32" E, with the elevation of 1521 m above the sea level, in the west of Iran. Map of the study area was drawn by Arc GIS version 10 (Figure 1C). The population of this city in 2014 was estimated to be 432 330. Twenty rodents were collected from different urban areas of Sanandaj in a period of more than 2 years.

Rodent Collection, Identification And Separation of *Capillaria hepatica* From Infected Liver

Four *R. norvegicus*, 6 *R. rattus*, and 10 *M. musculus* from the study area were captured using live trap each time in interval of 1 day between 2 weeks. The mice were

labeled and transferred to the laboratory of parasitology at the Kurdistan University of Medical Sciences, then sacrificed humanely by an overdose of ketamine and xylazine intraperitoneally injected, and identified based on diagnostic keys with regard to the proportion of the size of tail to whole body, ear to eye distance and dental pattern.¹⁸ After autopsy, we speculated whether the livers were infected with *C. hepatica*. Worms that had infected the livers were separated from the liver parenchyma using fine needles under stereomicroscope. Then, all the livers were separately homogenized, washed with PBS, and centrifuged at 1000 g at least three times, and obtained sediments were observed by a microscope.

Sectioning and Staining

One of the infected livers was processed and stained with hematoxylin and eosin based on a prior study^{19,20}. Briefly, one part of the liver was trimmed, fixed in 10% formaldehyde for 24 hours, dehydrated in each of the 50%, 70%, 90% and 2 series of 100% ethanol for 15 minutes. Then, clearing was done with 2 series of xylene for 20 minutes. Hot paraffin (50°C) was added to infiltrate the tissue for a sequence of two 30-minute periods and it was embedded carefully in paraffin cassettes to determine the plane of section. Sectioning was done with microtome in 2-4 mm thickness, and the sections were mounted on microscope slides. Then, they were stained with hematoxylin and eosin and finally the sections were examined under a light microscope.

Embryonation of the Eggs of *Capillaria hepatica*

Two sets of the eggs that were derived from infected livers were cultivated at first in potassium dichromate 2.5% and then in distilled water with 2% formaldehyde at room temperature until the eggs were embryonated.

Figure 1. Map of Sanandaj, west of Iran, area from where the rodents were collected. (A) Iran on the map of the world, (B) Kurdistan on the map of Iran, (C) Study area of Sanandaj on the map of Kurdistan, drawn by Arc GIS version 10.

Experimental Infection of Balb/c Mice With *Capillaria hepatica*

After observing motile larvae within the eggs, at least 30 eggs with motile larvae from the existing mix of eggs from cultivation in 2.5% potassium dichromate, distilled water and 2% formaldehyde were washed with normal saline and centrifuged at 500 g for 3 times. Then, they were administered orally to 3 six-week-old male Balb/c mice using gavage needles number 18. The first mouse was sacrificed 1 month after administration, the second was sacrificed 3 months after administration, and the third was sacrificed 9 months after administration of the eggs²¹

Results

Overall, 20 samples including 4 *R. norvegicus*, 6 *R. rattus* (Figure 2A) and 10 *M. musculus* were collected. *C. hepatica* was detected in 5 out of 20 livers (25%) (Figure 2B), and worms (Figure 2C) and eggs (Figure 2D) were separated from the livers. The liver infection was 50% (2/4) in *R. norvegicus*, 50% (3/6) in *R. rattus*, and 0% (0/10) in *M. musculus*.

Cross section showed active infection of the liver with eggs deposited beside the worm (Figure 3A) and even inside the female worms (Figure 3B). Furthermore, in another part of the same liver, a fibrous capsule was observed around unembryonated eggs that could be related to a prior infection with *C. hepatica* (Figure 3C). Due to hyper infection, the lesions were observed in different parts of liver lobes and in some cases the worms could not be separated intact from the liver.

Three days after oral administration of the eggs with motile larvae (Figure 2E), the mice were reluctant to move and were hunched, and their fur were ruffled. After autopsy, by gross and microscopy examination, signs of infection were observed in none of the infected Balb/c mice.

Discussion

This study was the first report on *C. hepatica* infection in *Rattus* spp. in Sanandaj, capital city of Kurdistan province, in the west of Iran. Due to the zoonotic importance of capillariasis and its potential in infecting humans, for

Figure 2. *Rattus rattus* With *Capillaria hepatica* Infection Captured in Sanandaj. (A) *R. rattus* captured in Sanandaj with macroscopic liver lesion, (B) Abnormal liver with rough different colored spots on the surface of the liver, (C) The adult worm of *C. hepatica* isolated from an infected liver was measured approximately 35 mm, (D) Brown oval shaped egg of *C. hepatica* with the size of approximately 55 microns and shallow polar knobs at both ends and double layered shell with minipores that can be seen in the outer shell of *C. hepatica*, (E) Motile larvae within the egg of *C. hepatica* after 39 days of cultivation in potassium dichromate 2.5% at room temperature.

Figure 3. Hepatic Tissue of *Rattus rattus* Infected With *C. hepatica*. H & E staining. (A) Eggs deposited beside the female worm and inside the worm, (B) Cross section of a worm inside liver parenchyma where eggs are inside the female worm. (C) Cluster of bioperculate eggs and fibrous tissue around the eggs.

example the true case report of a severe *Capillaria* infection in a 5-year-old child of Iran in 2015,⁷ and the fact that genus *Rattus* is considered a reservoir host for this infection, infected rodents are reported as risk factors for public health. Rodents' infection has been reported from different parts of Iran including Kermanshah in 23 *R. norvegicus* and 5 *R. rattus* with the infection rate of 13.04% and 20.0%, respectively.²² The infection was reported in Ardabil in 120 *Meriones persicus* with the infection rate of 6.9%²³.

The infection rates in some other parts of the world were 36% in captured *R. norvegicus* in Milan, Italy²⁴, 8% in 3 non-commensal rodent species from Geneva, Switzerland,²⁵ 87.9% in Norway rats in Baltimore, Maryland, USA,¹¹ and 35% of *R. rattus* and 54% of *R. norvegicus* in Australia²⁶ However in several studies in Iran, the researchers were not able to find *Capillaria* infection in their studies, for example in a study performed in Ahvaz,²⁷ as well as in another study conducted in Dezful and Andimeshk, in Khuzestan province²⁸ and also in wild rodents in Sistan and Baluchistan province.²⁹ This probably means that the endemic area of *C. hepatica* is confined to specific parts of Iran and is not distributed uniformly in all parts of the country. In order to determine the real status of these parasites, conducting more studies in other parts of Iran is required.

The rats captured for the study of *Capillaria* infection were infected similar to studies reported in other parts of the world, including Milan, Italy,²⁴ Baltimore, Maryland,³⁰ Brazil,³¹ Switzerland,²⁵ and Egypt.³² In addition, the rats captured from Sanandaj showed partial resistance to this nematode since they were heavily infected, without showing apparent clinical signs and had fibrous clusters of eggs in one part of their liver as well as active infection of live adult worms in other parts (Figure 3C). It can be concluded that the rats of these areas, in addition to being reservoir hosts for *C. hepatica* nematode, are also a potential risk factor to the inhabitants of Sanandaj. However, 10 house mice (*M. musculus*) captured from urban and suburban areas of Sanandaj, showed no evidence of *Capillaria* infection in their livers, and surprisingly we failed to infect Balb/c mice with embryonated eggs orally. Although some studies have shown *C. hepatica* in the liver of house mice and even named this nematode as a potential organism for biological control of mice plague through falling natality as well as decreased survival rate of mice from lactation period.^{33,34} In addition, another study showed *Capillaria* in the liver of house mice and considered house mice as a source of infection, similar to *Rattus* species³⁵ Though in some studies with regard to low prevalence of *Capillaria* spp. in *M. musculus*, it has been suggested that this species is less affected by *C. hepatica* and house mice, also, is rarely infected by it.³⁶ Studies carried out on *M. musculus* showed differences in the susceptibility of this species to *Capillaria* infection.

Findings of this study showed no infection with *Capillaria* spp. in house mice, which could mean house mice probably do not play an active role in the life cycle of this disease in Sanandaj.

The possible mechanism for the presence of infection in rats and no infection in house mice in this study might be related to various forms of behavioral patterns in these species, and conditions that separate community organization and population dynamics in these 3 species; so maybe none of them enters the territory of other species.³⁷ Another possible mechanism might be related to the genetic diversity of *C. hepatica* in different geographical regions, though proving this theory requires conducting molecular studies in this area.

Conclusion

The main reservoir hosts of *C. hepatica* in Sanandaj are *R. rattus* and *R. norvegicus*, and they could be considered as risk factors for public health. On the other hand, *M. musculus* that was included in this study was not infected experimentally with *Capillaria*, which probably means that no significant role for the infection or transmission of *Capillaria* could be considered for it; and unsuccessful attempts to infect Balb/c mice experimentally, with embryonated eggs orally, could comply with this claim, or at least should be carried out to validate this finding. Lastly, due to the high prevalence rate of *C. hepatica* in rodents of the study area, obscured liver syndromes particularly in children should be considered as suspicious cases of *Capillaria* infection by healthcare providers, and it is recommended investigating them via various methods, such as serological tests, biopsy and magnetic resonance imaging (MRI).

Ethical Approval

The study was supervised and approved by Ethics Committee of Kurdistan University of Medical Sciences for handling and anesthesia of animals sacrificed in the study.

Competing Interests

Authors declare that they have no competing interests.

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