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Dicrocoelium dendriticum found in a Bronze Age cemetery in western Iran in the pre-Persepolis period: The oldest Asian palaeofinding in the present human infection hottest spot region

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ABSTRACT

Dicrocoeliasis of animals and humans is caused by trematode species of the genus *Dicrocoelium*, mainly *Dicrocoelium dendriticum* in ruminants of the Holarctic region. *D. dendriticum* may be considered an old parasite, probably related to the appearance and diversification of Eurasian ovicaprines, occurred 14.7–14.5 million years ago. The oldest palaeoparasitological findings of *Dicrocoelium* in domestic animals and humans date from more than 5000 years BC in Europe. Eggs of *D. dendriticum* have been found in a burial of a Bronze Age cemetery (2600–2200 BC) close to Yasuj city, southwestern Iran. This is the oldest finding of *D. dendriticum* in the Near East, where present human infection reports are more numerous than in other world regions where human dicrocoeliasis is rare and sporadic. This palaeofinding in the Zagros mountainous chain area is of interest by its location close to Persepolis, suggesting a narrow relationship between humans and herbivorous animals in these highlands. Domestic ruminant populations of these highlands were following a repeated contact with those of the western flat lowlands of the Fertile Crescent thanks to annual altitudinal transhumance migrations of the nomadic pastoral tribes with their herds living throughout Zagros Mountains in the several millennium period BC. It is concluded that *D. dendriticum* spread together with sheep and goats westward throughout Europe from the Fertile Crescent during the 8000–6000 year BC period and somewhat later southward into Africa, both spreads facilitated by the low specificity of that trematode species regarding the snail and ant intermediate hosts.

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1. Introduction

Dicrocoeliasis is a trematodiasis caused by digenean species of the genus *Dicrocoelium* (*Trematoda*: *Dicrocoeliidae*), mainly *Dicrocoelium dendriticum* (=*Dicrocoelium lanceolatum*) throughout and *Dicrocoelium hospes* in Africa southward from the Sahara. The adult stage is a parasite of the bile ducts and gall bladder, of a relatively small size of about 8–14/2–2.5 mm, and which is commonly known as the lancet fluke, the lanceolate fluke, the little liver fluke or the small liver fluke. It shows a scarce definitive host specificity, although with pronounced preference for ruminants which may be considered the true original hosts, among which mainly mid-sized ruminants as sheep and goats and secondarily large ruminants as cattle. Their transmission involves a three host life cycle, with embryonated eggs of 35–45/22–30 μm expelled with the mammal feces, to follow a completely terrestrial life cycle. Many terrestrial snail species act as first intermediate hosts, with differences depending on the geographical area. Several ant species, mainly belonging to the genus *Formica*, act as second intermediate hosts inside which the infective metacercariae develop. The ruminant hosts become infected by ingesting these ants when grazing [1].

Infection by *D. dendriticum* is an important parasitic disease in animals from an economic and health viewpoint [2]. In humans, clinical symptoms of true dicrocoeliosis are neither uniform nor specific. As with other liver fluke infections, the pathology depends on the number

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of flukes present and the duration of the infection. Because of the small size of the fluke and its smooth and spineless surface, mechanical and toxic damages are much less than in fascioliasis and opisthorchiasis [1]. Moreover, the main route of migration of *D. dendriticum* to reach the liver is via the bile ducts, instead of via the abdominal cavity route as in *Fasciola hepatica* [3]. Highly pathogenic infections in humans have been however described, as rare neurological symptoms and subsequent meningeal syndrome [3,4] and recently also involvement in an allergic pharyngitis syndrome known as "Halzoun" [5]. Humans acquire the infection accidentally, by swallowing an infected ant together with the food, such as vegetables, fruits, etc., while staying in an endemic area.

This parasite species has a more or less cosmopolitan distribution in herbivorous mammals, mainly in ruminants of the Holarctic region. The parasite is believed to have been introduced into the New World with the extensive importations of dairy cattle from European countries. Human infections have been recorded in *D. dendriticum* enzootic areas. Although the majority of these infections are of rare and sporadic occurrence, they are undoubtedly underestimated. Most infections are only spurious, the appearance of eggs in stools being due to the ingestion of infected livers of sheep, goat and cattle, eggs being detected in feces for only a few days. The reason for the availability of infected animal livers on the market is that not all infected livers show signs of the infection. Genuine human infection can be verified through parasite finding in surgical operations, evidence of permanent egg shedding through time, egg recovering in duodenal aspirates, or the existence of related symptomatology. Human infection has been reported from Asia, northern Africa, Europe, North America and South America [1].

D. dendriticum may be considered an old parasite, probably related to the appearance of Eurasian ovicaprines. The diversification of Caprinae occurred 14.7–14.5 million years ago (mya) and the divergence of *Ovis* and *Capra* 11.3 mya, the later species diversification within these two genera having been dated around 5.1 to 6.8 mya [6]. Wild herbivore domestication began around 10,000 years ago at the dawn of the Neolithic in the region known as the Fertile Crescent, a formerly fertile, now partly desert, area in the Near and Middle East which was an agricultural region extending from the Levant (lands bordering the Eastern shores of the Mediterranean and Aegean seas) eastward including modern-day Israel, Jordan, Lebanon and western Syria, into southeast Turkey and, along the Tigris and Euphrates rivers, into Iraq and the western flanks of Iran [7–10]. The Neolithic period was an era of major changes in human life. Goats, in the form of their wild progenitor the bezoar *Capra aegagrus*, also called Persian wild goat [11], were the first wild herbivores to be domesticated. Sheep and goat domestication played an important role in the phenomenon of Neolithization occurring in the late prehistory of the Near and Middle East between 9000 and 5000 BC. Data from the numerous neolithic human settlements found throughout this region strongly point to it as a major domestication center for livestock species, mainly goats and sheep [12]. Ovicaprine domestication gave rise to the transition from a nomadic to a sedentary way of life [13], human life becoming close to that of these mid-sized and readily mobile ruminants and thus also to their parasites.

The oldest record of *Dicrocoelium* was made in mammal coprolites dated from the Middle Pleistocene in France, 550,000 years BP [14]. Palaeoparasitological findings of *Dicrocoelium* do afterwards only appear in sites of 5259 years BC in Germany and in 3917–1750 years BC in France, Germany, Switzerland, in 3365–2600 years BC in Sudan and Iron age in South Africa, and later, already in different centuries AD in these European countries plus Austria and England [15], and also in the 17th century in Canada [16].

The present study deals with the palaeoparasitological finding of *D. dendriticum* in an archeological remain in the neighborhood of the city of Yasuj, in southwestern mountainous Iran. Besides the description of the egg materials found, focus is made on the closeness with the ancient city of Persepolis, the ceremonial capital of the Achaemenid Empire, and the interesting relationships of the mountainous area in question with the western flat lowlands of the Fertile Crescent by means of nomadic tribes practicing altitudinal transhumance with their herds.

2. Materials and methods

Specimens including soil samples obtained from three burials and one burial jar were collected during a rescue excavation in a Bronze Age cemetery (2600–2200 BC) located in the neighborhood of the city of Yasuj, Kohkilooye and Boyer-Ahmad province, in southwestern Iran, in the late winter 2012. The archeological remains are located at 70 km northwestward of the aforementioned city, at the coordinates of 31°9′13″ N and 51°6′14″ E (Fig. 1). Excavation procedures were undertaken in a way to prevent burial materials to be contaminated with external soil.

Archeological dating was comparatively proved, based on potteries and bronze items found in the graves. For the retrieving of helminth eggs, the rehydration technique in 0.5% trisodium phosphate was used, according to the method successfully practiced so far [17]. Recovered eggs were photographed, measured and identified morphologically, according to available references.

3. Results

A total of 1100 slides from rehydrated soil samples were thoroughly investigated under the light microscope. During this careful microscopic investigation, 750 slides were also thoroughly examined for the eventual detection of helminth eggs.

Two eggs showing typical trematode features could be identified in grave N2 (Fig. 2). When microscopically measured, these eggs proved to be brown, thick-shelled and with an inconspicuous operculum, of a size of 38.9/23.0 μm of length/maximum width, in an excellent conservation condition and even allowing for the distinction of miracidium remains inside.

4. Discussion

The characteristics and size of the trematode eggs found fully agree with those of the eggs of the species *D. dendriticum*, which are known to have a length/width range of 35–45/22–30 μm [18]. The finding in the northern hemisphere allows for the ruling out of the other species also infecting humans, *D. hospes*, with similar eggs but restricted to the African continent [1].

In Asia, there are no palaeoparasitological findings of *Dicrocoelium* [19,20], except the only very recent one in an ancient salt mine in Chehrabad, northwestern Iran, around 2500–1500 years BP [21]. Our finding becomes thus the oldest one of *D. dendriticum* in Asia and the second palaeoparasitological finding in Iran.

This report being the oldest palaeoparasitological finding of *D. dendriticum* in Iran is somehow surprising, as indeed the Near East (Fig. 1) is the only region of the world in which present human infection reports are clearly more numerous than in other regions where human dicrocoeliasis only appears to be rare and sporadic.

In Turkey, in the last century a report referred seven mature *D. dendriticum* in a 26-year-old woman after a gall bladder operation [22] and this trematode was found in 0.96% among 6311 patients [23]. Other reports in Turkey were published in the first decade of the present century [24–32], of which several reported severe pathogenicity [28,31]. Saudi Arabia stands out by the high number of patients diagnosed expelling eggs in their feces [33–37]. In Iran, reports refer to human infection in different parts of the country and since long time [38–40]. This human dicrocoeliasis hot spot area may be even considered to be spread more eastward, as suggested by the finding of *D. dendriticum* adults in the livers (up to 4 flukes/liver) of 37 of the 13,287 corpses autopsied from 1968 to 1986 in Uzbekistan, in which infection was reported to

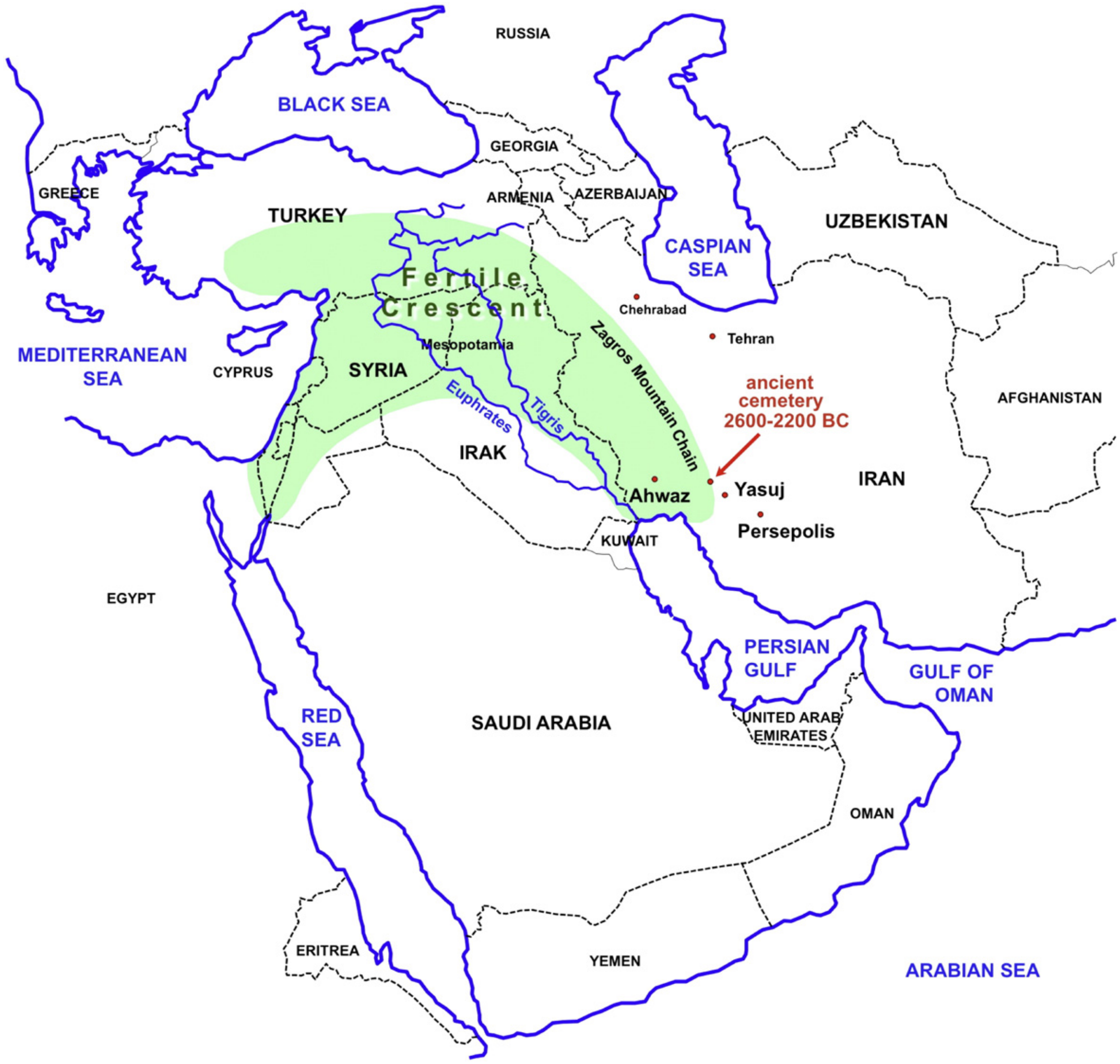

Fig. 1. Map of the Near East showing the location of the Bronze Age cemetery in the neighborhood of the city of Yasuj, in Iran, where *Dicrocoelium dendriticum* eggs were found in a grave, geographical distribution covered by the ancient Fertile Crescent around the rivers Euphrates and Tigris, and present countries included in the *Dicrocoelium dendriticum* human infection hottest region of nowadays. Note geographical closeness of aforementioned cemetery to Persepolis and Ahwaz.

Fig. 2. Egg of *Dicrocoelium dendriticum* found in a grave of a Bronze Age cemetery, dated 2600–2200 years BC, in the neighborhood of the city of Yasuj, in Iran.

be the cause of death in no case, nor was the infection ever diagnosed during life [41].

The presence of *D. dendriticum* since thousands of years, as indicated by the palaeoparasitological finding of our report, may be interpreted as a parasite being well established and widely distributed in that region. Consequently, additional palaeoparasitological findings of *D. dendriticum* in future archeological explorations, whether in domestic livestock or in humans, might be expected, even older than the one described in this study.

This hypothesis is also supported by the widespread dicrocoeliasis in Iranian ruminants [42,43], as well as by the low specificity of *D. dendriticum* at the level of terrestrial snail host and ants second intermediate hosts (different widespread species of *Formica* and related genera) [44–46]. Indeed, the principal human source of dicrocoeliasis becomes infected sheep with extremely high worm loads (up to 108,000 worms), and a very high level of incidence of infection (often 100%). The consequence is a high rate of pasture infestation resulting from fecal contamination, which is in turn potentiated by large-scale, intensive breeding of these animals. The great quantity of eggs laid by a single fluke during its several-year life-span is multiplied many times by the larval stage multiplication at snail host level. This is responsible for the

Fig. 3. Stone wall illustrations of life scenes including domesticated mid-sized and large sized ruminants in the present ruins of Persepolis, the ancient ceremonial capital of the Achaemenid Empire, dated from around 515 BC.

heavy infestation of the pasture environment, especially under favorable conditions [1].

Additionally, this *D. dendriticum* egg finding in the neighborhood of Yasuj, in the Zagros mountainous chain area, is of special interest by its geographical location, which appears to be (i) southward close to Persepolis and (ii) westward close to the original Fertile Crescent flat lowlands.

Persepolis is located at 1630 m high altitude and only around 150 km southeast of Yasuj. The earliest remains of this ancient city date from around 515 BC, that is, around 2000 years later than the cemetery discovered at 1500 m high altitude close to Yasuj, a city which is nowadays located at 1800 m altitude (Fig. 1). However, Persepolis archeological ruins, located at 1630 m high altitude, offer interesting reliefs, illustrating animal domestication throughout the highlands of the Zagros mountainous chain area. Thus, sheep, goats and zebu cattle (Fig. 3), the main definitive hosts of *D. dendriticum*, but also other potential hosts such as donkeys, horses, camels and dromedaries [1], appear repeatedly in the scenes illustrated on the stone walls of the ancient Persepolis buildings. This speaks about a close relationship between humans and these herbivorous animals also in the highlands in question. The geographical proximity of the Yasuj palaeofinding and Persepolis highlights the common feature of an altitude area, which is of great importance for the life cycle and snail vectors of trematodes [12].

Additionally, all indicates that the domestic ruminant populations of these highlands were following a more or less continuous contact with those of the western flat lowlands of the Fertile Crescent thanks to annual altitudinal transhumance migrations (from the lowlands in cold months to the highlands in hot months) of nomadic pastoral human groups with their herds coexisting with sedentary people living in permanent Neolithic settlements throughout the Zagros mountainous chain area in the several millennium period BC [47–50]. Several of these nomadic tribes (Boyerahmadi, Bahmaei, Qashqai and Bakhtiari) are still practicing altitudinal transhumance in the Iranian province of Yasuj city (Kohkilooye and Boyer Ahmad) and neighboring provinces (Khuzestan, Chahar Mahaal and Bakhtiari, Isfahan, and Fars) nowadays. The presence of *D. dendriticum* in the western lowland area of Ahwaz (Khuzestan province) [43] is an additional support to the aforementioned considerations (Fig. 1).

Archeozoological analyses showed that sheep and goats had been imported into the Euphrates mid-valley already around 8200–8000 years BC [51]. Evidence by ancient DNA analysis of 8000 BC near eastern farmers has supported an early neolithic pioneer maritime colonization of mainland Europe through Cyprus and the Aegean islands [52]. Therefore, it may be concluded that *D. dendriticum* spread together with sheep and goats westward throughout Europe from the Fertile Crescent during the 8000–6000 year BC period and somewhat later southward into Africa, both spreads facilitated by the low specificity of that trematode species regarding the snail and ant intermediate hosts. This agrees with the palaeoparasitological review on *Dicrocoelium* data from domestic livestock and humans throughout Europe and Africa [15]. Hence, it may be concluded that the oldest record of *Dicrocoelium* in mammal coprolites dated from the Middle Pleistocene in France, 550,000 years BP [14], was indeed not involving *D. dendriticum* but other dicrocoeliid species.

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