Dicrocoelium in Iran: From Bronze Age to the Twenty-First Century

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Abstract

Members of the genus *Dicrocoelium* are cosmopolitan hermaphroditic trematodes, which commonly occur in the biliary system of ruminants, especially cattle and sheep. There are two intermediate hosts (land snails and ants) involved in the parasite life cycle, and the final host becomes infected after ingestion of the larvae containing ant. Light infections are generally symptomless; therefore, the disease is often get underestimated by practitioners and researchers. In humans, *Dicrocoelium* causes a rare food-borne zoonotic disease of the biliary tract. In this chapter, we summarize the current knowledge on different aspects of *Dicrocoelium* and dicrocoeliosis/dicrocoeliasis, including biology, clinical presentations, pathogenesis, and diagnosis with special emphasis on the current and ancient epidemiology of this fascinating worm in Iran, covering 15 human cases from the Bronze Age to very 2022.

Keywords

Archaeology · *Dicrocoelium* · Dicrocoeliosis · Dicrocoeliasis · Iran · Paleoparasitology

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10.1 Introduction

10.1.1 What Is Dicrocoelium?

The members of genus *Dicrocoelium* Dujardin, 1845 (Greek *dicroos* = double, *koilia* = body cavity) are hermaphroditic trematodes normally found in the biliary system of ruminants, mainly cattle and sheep. *Dicrocoelium* species (e.g., *D. dendriticum*, *D. hospes*, and *D. chinensis*) are known as "small liver fluke, lancet fluke, and lanceolate fluke" and have low host specificity; thus, many other mammals such as camels, equids, rabbits and hares, dogs, and non-human primates can become infected. Humans are inadvertent definitive hosts (Otranto and Traversa 2003).

Five species, i.e., *D. dendriticum* Rudolphi 1819, *D. hospes* Looss, 1907, *D. chinensis* Tang and Tang, 1978, *D. orientale* Sudarikov & Ryjikov, 1951, and *D. petrowi* Kassimov, 1952, have been referred as valid (GBIF 2022). However, the most well-known one is *D. dendriticum* (Latin: *dendriticus* = furcated), with a global distribution in herbivorous mammals, mainly ruminants, of the Holarctic region. It is found in almost every country in the European continent and neighboring islands as well as the northern coast of Africa. In Asia, *D. dendriticum* is reported from Russia, Turkey, Syria, Iran, India, China, the Philippines, and Japan. In the Americas, the parasite is found in the USA, Canada, Cuba, Colombia, and Brazil. The second species, *D. hospes*, is endemic to sub-Saharan West Africa. The third well-known species, *D. chinensis*, has been mostly found in various ruminants from East Asia and in sika deer (*Cervus nippon*) from different regions of Europe, which is thought to probably be imported from Asian countries (Otranto et al. 2007).

10.1.2 Life Cycle

The life cycle of *Dicrocoelium* is a rather fascinating example of biological adaptation of a parasite to its host (Fig. 10.1). First intermediate hosts are various air-breathing land snails, such as species of the genera *Cochlicopa* (=*Cionella*), *Helix*, *Xerolenta* (=*Helicella*), and *Zebrina*. Over 90 species of land snails have been found to be competent vectors of *D. dendriticum*, several of which, particularly, *Cochlicopa lubrica*, have worldwide distribution. *Helicella obvia* is found in Germany, *Helicella corderoi* in Spain, *Zebrina hohenackeri* in the Caucasia, and *Cernuella virgata* in Italy. The cercariae are squeezed out from snails as "slime balls," which are clusters of many hundreds covered by a mucilaginous substance and are ingested by several species of ants, especially the members of genus *Formica*, e.g., *F. fusca*, *F. pratensis*, and *F. rufibarbis*, as the second intermediate host. The definitive mammalian host is infected accidentally via ingestion of an infected ant while feeding on vegetables, fruits, and water (Otranto and Traversa 2002; Toledo and Fried 2019).

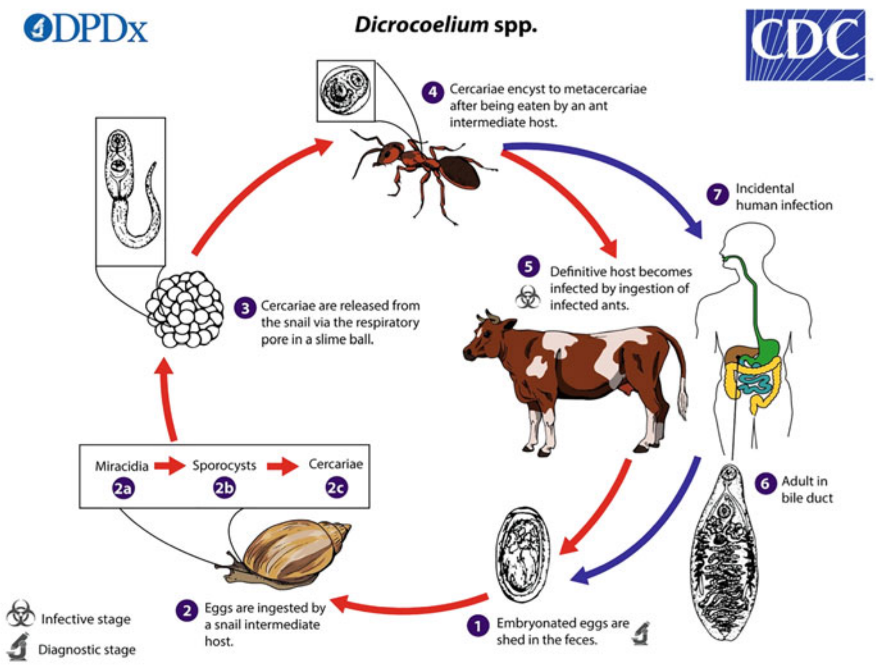

Fig. 10.1 Life cycle of *Dicrocoelium* spp. Dark brown embryonated eggs containing miracidia are shed in definitive hosts' (typically ruminants) feces (1). The operculated eggs, which are oval and unilaterally flattened and contain a miracidium, are then ingested by the snail as the first intermediate host (2). Upon hatching, the emerged miracidia (2a) penetrate the intestinal epithelium, migrate to the hepatopancreas and stay in the neighboring vascular connective tissue, and become mother sporocysts (2b). The sporocysts then migrate to the digestive glands where they produce several daughter sporocysts, each of which gives rise to numerous cercariae (2c). The cercariae migrate to the snail respiratory chambers, where they are shed from the host in masses of 3000–5000 cercariae, namely slime balls of 10 mm in diameter, which are enveloped in a mucilaginous substance (3). Upon ingestion of slime ball by the second intermediate host (ant), the cercariae encyst in the gaster becoming infective metacercariae. However, one (or rarely two) non-encysted cercaria invades and occupies the subesophageal ganglion (brain-worm), which can cause a cataleptic spasm at the ambient temperature of about 15–20 °C or lower (e.g., early in the morning). These spasms can "paralyze" the ant on the tips of pasturage and grass, where grazing ruminants can readily swallow it (4). When the infected ant is ingested by a proper definitive host (5), the metacercariae emerge from the cyst in the duodenum. The larvae migrate to the small and then larger bile ducts and finally to the gall bladder via the ductus choledochus, where they mature into egg-producing flukes (6). Humans can serve as definitive hosts if the infected ants are ingested (7). After approximately two months prepatent period during which reproduction by hermaphroditism or cross-fertilization takes place, the eggs are released into the environment via the host feces (Otranto and Traversa 2003; Toledo and Fried 2019). Image is reproduced from DPDx CDC https://www.cdc.gov/dpdx/dicrocoeliasis/index.html

10.1.3 Epidemiology in Livestock

The environment and presence of intermediate and definitive hosts play an important role in the epidemiology of *Dicrocoelium*. The presence of *D. dendriticum* depends upon dry and alkaline or calcareous soils, which provide ecologically favorable environments for its intermediate hosts. The parasite eggs are highly resistant in such an environment, as they can spend the winter and remain infectious for even 20 months on pasture. In countries of the Mediterranean region, the excretion of eggs in sheep feces is seasonal, with a winter peak of about 323.4 $\pm$ 18.5 eggs per gram (EPG). In addition, the epidemiology of dicrocoeliosis is influenced by: (1) host species, the susceptibility of sheep is higher than goats; (2) age of the animal, the prevalence of infection in cattle older than 6 years is higher than of calves younger than 18 months; (3) physiological condition, hosts stressed by confinement and transportation have higher levels of fecal egg excretion; and (4) sex, female animals show higher prevalences of dicrocoeliosis than males at necropsy. Differences in the dicrocoeliosis prevalence are also thought to depend on the snail species and the season; for instance, in Spain, a higher prevalence of infection was reported in September in *H. itala* and in February in *H. corderoi* than other months (Otranto and Traversa 2003; Manga-González et al. 2001).

10.1.4 Clinical Presentation in Livestock

Light infections remain largely symptomless; thus, the disease is often underestimated by practitioners and researchers because dicrocoeliosis is cloaked by the pathological effects of other parasitic infections. However, developmental delay may occur as a result of reduced liver activity. Heavy infections may lead to liver cirrhosis following cholangitis, granulomatous reaction, and abscess formation. Animals suffering from *Dicrocoelium* infection may show anemia, dropsy, and cachexia. Major hepatic lesions are detectable by examination of the organ during necropsy, through which superficial scars and disruption of bile ducts resulted from the buccal stylets of liver flukes are apparent. Most of the time, the infected liver is not consumable by humans and is condemned, thus imposing considerable economic losses to the livestock industry (Otranto and Traversa 2003).

10.1.5 Human Dicrocoeliasis

Both *D. dendriticum* and *D. hospes* cause rare food-borne zoonotic diseases in the human biliary system. Infection happens via the ingestion of metacercariae-containing ants, while pseudo-infections (presence of parasite eggs in feces in the absence of adult flukes) occur after the consumption of parasitized animal liver. Up until 1982, approximately 300 cases were documented in the literature based on the recovery of eggs in human feces; however, the epidemiology of human dicrocoeliasis is not known, especially when there are both true and pseudo-infections (Jeandron et al. 2011; Moure et al. 2017; Lavilla-Salgado et al. 2021). *Dicrocoelium dendriticum* is known to be present in definitive hosts throughout Europe, North Africa, and Asia and can be found infrequently in North America. The known clinical cases have mostly occurred in North Africa and the Middle East. On the other hand, *D. hospes* is endemic in sub-Saharan West Africa; several animal cases have been reported in Senegal, Ghana, Sierra Leone, and Mali. Most infections involve low numbers of worms and are not associated with observable symptoms. In severe infections, there may be symptoms of generalized gastrointestinal/abdominal distress associated with cholecystitis and liver abscesses. In addition, the existence of flukes in subcutaneous masses has been sporadically reported (https://www.cdc.gov/dpdx/dicrocoeliasis/index.html).

10.1.6 Diagnosis

In livestock, considering the subclinical nature of the disease, dicrocoeliosis is mostly diagnosed during necropsy of the liver, microscopic examination of duodenal fluid, and by coproscopy for the detection of parasite eggs. Adult *Dicrocoelium* is flattened, with both the anterior and posterior ends tapered. The paired testes are located behind the ventral sucker (acetabulum). The tiny ovary lies just behind the testes (Fig. 10.2a). The operculated thick-shelled dark brown eggs of *D. dendriticum* measure 35–45 μm × 20–30 μm and are fully embryonated when shed in feces (Fig. 10.2b).

In human patients, the adult flukes are hardly recovered, and eggs may be found in stool following consumption of the infected liver; therefore, additional samples should be collected to recognize this type of passage (aka, pseudo-parasitosis) from a true infection. Misdiagnosis can be avoided through stool examination following a liver-free diet for at least 3 days. Other differences between the true- and pseudo-infections are clinical manifestations, eosinophilia, elevation of IgE, liver and pancreas malfunction, existence of parasites eggs after a several days of

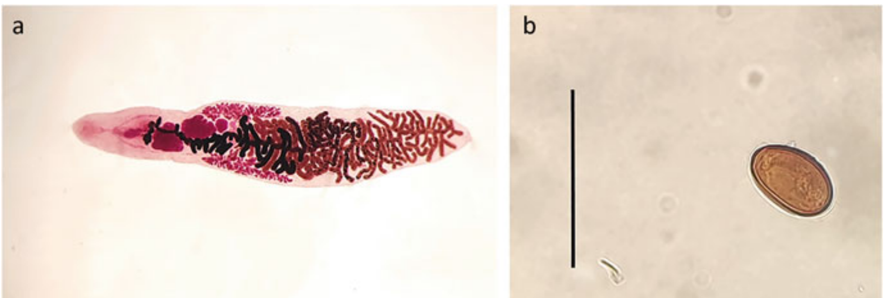

Fig. 10.2 (**a**) Adult *D. dendriticum*, stained with carmine. (**b**) Egg of *D. dendriticum* extracted from a mature trematode. Image courtesy of Sina Mohtasebi (University of Calgary, Canada), scale bar = 80 μm

consumption of liver free diet, and observation of embryonated eggs (Lavilla-Salgado et al. 2021). The most common laboratory finding is "eggs in transit" with a variable color shell of yellow, orange, or light brown, containing an indistinct dark yellow oval mass, often with 1–4 shiny globules. Embryonated eggs, instead, exhibit a uniformly dark brown shell, containing a ciliated embryo (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5303036/figure/fig1/) (Engbaek et al. 2003).

10.2 Ancient Dicrocoeliosis/Dicrocoeliasis

10.2.1 What Do We Know?

The existence of ancient dicrocoeliosis/dicrocoeliasis is confirmed based on the discovery of eggs preserved in ancient human and animal coprolites or in archaeological samples from occupation layers, which are then commonly extracted using micrometrical sieving techniques. The most ancient *Dicrocoelium* eggs belong to earlier than 550,000 years BP (Pleistocene period) and were recovered by analysis of a coprolite sample from "Caune de l'Arago," a cave near Tautavel, France (Jouy-Avantin et al. 1999), indicating the long time presence of dicrocoeliosis/dicrocoeliasis in the Old World. Studying the paleoparasitological records, Le Bailly and Bouchet (2010) chrono-geographically classified the occurrence, distribution, and migration evidence of ancient dicrocoeliosis/dicrocoeliasis. They discussed relationships between ancient human/animal migrations and the presence of *Dicrocoelium* in the archaeological areas of the Old World and New World. In addition, they noted that the *Dicrocoelium* findings could represent herding and breeding and probably dietary habits of ancient humans over the world (Le Bailly and Bouchet 2010). Available paleoparasitological findings in neighboring regions of the Middle East indicate that the trematode eggs found in the archaeological sites of the European continent are largely overtopped by the members of class Trematoda: *Fasciola* and *Dicrocoelium* (Bouchet et al. 2003; Dittmar and Teegen 2003). Moreover, there is only one report of a single ancient fluke egg (*Dicrocoelium* sp.) from South Africa (Dittmar and Steyn 2004).

The paleoparasitological recovery of several *Dicrocoelium* sp. eggs in Kerma, Sudan, could provide the first bit of information on ancient dicrocoeliasis in the Middle East (Harter 2003). In another survey, Zias and colleagues found *Dicrocoelium* sp. eggs in a soil sample from an ancient animal stable, northwest of the Qumran archaeological site in the Middle East (Zias et al. 2006).

A review by Savinetsky and Khrustalev presents findings of parasites through examination of ancient animal dung layers and coprolites in northern neighboring regions of the Middle East, Russia, North Caucasus, and middle Asia (South Turkmenistan). The authors found *Dicrocoelium* eggs in deposits of sheep dung (*Ovis aries*) and East Caucasian turs (*Capra caucasica cylindricornis*) (Savinetsky and Khrustalev 2013).

10.2.2 Challenges in Ancient Egg Identification

The use of light microscopy in paleoparasitological research is problematic, when applied for egg identification, since many parasites from the same genus have eggs with very similar shapes and sizes. This similarity sometimes occurs in eggs of the same family and even the same order. Identification of eggs is more precise when the biological origin of the studied material is known. However, many analyses are performed on sediment samples collected from latrines or cesspits; thus, the biological origin of the fecal material from such structures may have single or multiple origins (Le Bailly and Bouchet 2010). In such cases, identifications are less precise and are the subject of debate (Sazmand 2021).

It has been stated that "*the identification of the eggs* as D. dendriticum *for the majority of all the European paleoparasitological results is almost certain since all other species have a different current geographical distribution and/or a different egg size*" (Le Bailly and Bouchet 2010). Based on this assumption, *Dicrocoelium* spp. eggs detected in coprolites in Iran were reported as *D. dendriticum* (Makki et al. 2017; Bizhani et al. 2017; Mowlavi et al. 2015). However, given the present dearth of information on the archaeomalacology and lack of molecular studies, other present or extinct *Dicrocoeliidae* species, e.g., *D. chinensis* or *D. hospes*, cannot be ruled out. In this instance, the role of climate changes in geographic distributions of snail-borne trematodes would need to be considered in the differential diagnosis of the recovered eggs (Stensgaard et al. 2019). Thus, it becomes evident that further study of mollusks in the archaeological sites is required for recognition of possible sources of trematode infection in the given area (Insoll and Hutchins 2005).

10.3 Dicrocoeliosis and Dicrocoeliasis in Iran

10.3.1 Current Epidemiology

In livestock, *D. dendriticum* dicrocoeliosis is widespread all over the country (Nazarbeigy et al. 2021). Adult worms and/or eggs have been found in sheep, goats, cattle, buffaloes (Borji et al. 2012; Rafyi et al. 1971), camels (Sazmand et al. 2019; Sazmand and Joachim 2017), horses, and donkeys (Sazmand et al. 2020). Previous epidemiological studies showed infection rates of up to 85% in sheep, 66% in cattle, and 23.25% in goats (Ahmadi et al. 2010). Based on a systematic review of articles published between 2000–2015, 3.1% (2.2–4.2%) of sheep, 1.3% (0.9–1.9%) of goats, and 2.1% (1.1–3.5%) of cattle were infected with *D. dendriticum* in Iran (Bari et al. 2016). According to a more recent article, abattoir data were collected from 413 abattoirs representing all 31 Iran provinces (Kiani et al. 2021). In total, 3.08% (95% CI: 3.07–3.09%) of slaughtered cattle and 4.63% (95% CI: 4.62–4.63%) of slaughtered sheep and goats were infected with *Dicrocoelium* spp. from 2015 to 2019. Annual prevalence of cattle dicrocoeliosis ranged from 2.7% in 2019 to 3.0% in 2015, while in sheep and goats it ranged from 3.7% in 2015 to 4.8% in 2019. The majority of dicrocoeliosis cases were found in northern Iran, which has a Mediterranean climate. Considering the average price of US$8.20 per cattle liver and US$15.24 per sheep/goat liver, condemnation of 186,009 cattle and 2,701,274 sheep and goat livers has resulted in economic losses of US$1,668,986 and US$41,771,377, respectively (Kiani et al. 2021).

In wild mammals, there are also a few reports of dicrocoeliosis. The infection of *Ovis ammon orientalis* (wild sheep), *Gazella subgutturosa* (Goitered gazelle), and *Sus scrofa* (wild boar) has been documented (Eslami and Farsad-Hamdi 1992; Eslami et al. 1980).

In humans, 14 confirmed cases of dicrocoeliasis have so far been reported from Iran, all of which except three were from the Northern provinces of Mazandaran, Gilan, and Golestan. In these areas, the local population generally consumes large quantities of raw vegetables, both as appetizers and as part of their main meals. The first two patients were diagnosed with the infection in 1967 following an examination of 1065 individuals in Mazandaran (Sohrabi 1973). The next patient was a 15-year-old boy from Isfahan, central Iran, and was detected during coprological examination of 718 individuals (Farid 1971). Arfa and his colleagues in 1977 reported seven patients during the examination of 1240 individuals in Golestan province (Arfaa et al. 1977). There were no reported human cases for decades until 2010, when two patients were diagnosed in Gilan province (Ashrafi 2010). In the same year, one patient suffering from chronic diarrhea was diagnosed to be infected with *Dicrocoelium* in Esfahan (Mahmoodi et al. 2010). In another occasion, one individual from Qazvin was found positive for *Dicrocoelium* egg in the fecal specimen (Sadeghi and Borji 2015), but the authors did not follow up on the case to find out if he/she was a true-infected case. Very recently in 2022, another patient, again in Gilan, has been diagnosed with the infection (personal communication with Professor Keyhan Ashrafi).

10.3.2 Archaeoparasitological and Paleoparasitological Findings

Since 2013, a number of paleoparasitological reports have been published from archaeological sites in Iran, among which are the records of fluke ova, including *Dicrocoelium* sp. eggs in samples belonged to ancient Iran from the Bronze Age to Sassanid archaeological strata (Askari et al. 2018; Bizhani et al. 2017; Makki et al. 2017; Mowlavi et al. 2015; Nezamabadi 2014; Nezamabadi et al. 2013). All these findings are based on egg recovery using a trisodium phosphate solution for rehydration of soil sediments and observation of samples by light microscopy.

The first case of ancient *Dicrocoelium* egg in Iran was reported by Nezamabadi et al. (2013) following an examination of fecal polluted soil sediment without definite dating, but recovered from the archaeological layers of Chahrabad salt mine in North Western Iran. Therefore, this record should not be considered reliable paleoparasitological data due to incomplete description and inaccurate dating of the main sediment sample. In particular, the structure of the salt mine under archaeological excavation raises the possibility of contamination of deeper/older layers by more superficial ones belonging to more recent periods. The only *Dicrocoelium* egg recovered in 2017 from the Kiasar archaeological site, dated back to 247 BC–224 AD (Bizhani et al. 2017), also seriously lacks the essential criteria for being identified as a parasite egg. The image provided in this article is not clear enough, potentiating the risk of confusing pollen grain with *Dicrocoelium* egg (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5558072/figure/F3/). Another single *Dicrocoelium* egg reported in 2017 was collected from the Shahr-e Sukhteh site, located in southeastern Iran and dated back to 3200–1800 BC (Makki et al. 2017). Similarly, this report suffers from the same shortcomings, raising doubts about the correctness of *Dicrocoelium* egg identification (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5450963/figure/f3-kjp-55-2-197/). However, up until now, it seems that the presence of two *Dicrocoelium* eggs extracted from a burial sample in a Bronze Age cemetery, dated 2600–2200 BC in Yasuj, southwestern Iran (Mowlavi et al. 2015), is the only reliable record of the presence of *Dicrocoelium* in ancient Iran.

10.4 Conclusion

This chapter introduced the current knowledge on different aspects of *Dicrocoelium* and dicrocoeliosis/dicrocoeliasis, including biology, clinical presentations, pathogenesis, and diagnosis, with special emphasis on the current and ancient epidemiology of this fascinating worm in Iran. Since Iran rests along the Silk Road, a network of trade routes that connected the East and West, it was central to the economic, cultural, political, and religious interactions among these regions from the second century BC to the eighteenth century AD. Furthermore, the present ruins of Persepolis, the ancient ceremonial capital of the Achaemenid Empire (dated from around 515 BC), show that conquered peoples of different nations, including Ethiopians, Arabs, Thracians, Indians, Parthians, Cappadocians, Elamites, and Medians, brought gifts and animals from different nations for the king (Fig. 10.3). Hence, until now, we cannot determine the origin of *Dicrocoelium* in Iran. Advancement of ancient DNA analysis employing molecular phylogenetic techniques might shed light on the infection dynamics throughout history.

Fig. 10.3 The panels at the southern end of the Apadana Staircase in Persepolis show 23 delegations bringing their tributes, such as animals, to the Achaemenid king. Among them, Ethiopians, Arabs, Thracians, Indians, Parthians, Cappadocians, Elamites, and Medians have been recognized. Human and animal movements could have impacts on the transfer of pathogens such as *Dicrocoelium*. Pictures are reproduced from Wikimedia Commons files licensed under the Creative Commons Attribution 2.0 Generic license

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