Dicrocoelium dendriticum: An Unusual Parasitological Diagnosis in a Reference International Health Unit

Zaira Moure,^{1*} Francesc Zarzuela,^{1,2} Mateu Espasa,^{1,2} Diana Pou,² Nuria Serre-Delcor,² Begoña Treviño,² Cristina Bocanegra,² Israel Molina,^{1,3} Tomas Pumarola,¹ and Elena Sulleiro^{1,2}

¹Department of Microbiology, Vall d'Hebron University Hospital (HUVH), Universitat Autònoma de Barcelona, PROSICS, Barcelona, Spain;

²Tropical Medicine Unit Vall d'Hebron-Drassanes, Universitat Autònoma de Barcelona, PROSICS, Barcelona, Spain; ³Department of Infectious Diseases, Vall d'Hebron University Hospital (HUVH), Universitat Autònoma de Barcelona, PROSICS, Barcelona, Spain

Abstract. Finding *Dicrocoelium dendriticum* eggs in human feces is exceptional and there are few prevalence data available. True infection occurs after accidental ingestion of ants containing metacercariae and spurious infection through the consumption of infected animal liver. Differential diagnosis between true and pseudo-infections is performed through stool examination after a diet free of liver. In addition, microscopy can help to differentiate the type of infection. We report six cases, all from sub-Saharan Africa, detection of this fluke at the Tropical Medicine Unit Vall d'Hebron-Drassanes (Barcelona, Spain). *Dicrocoelium dendriticum* transit eggs were visualized in five cases and there were no subsequent visualizations after diet, which reinforces that all these cases were false parasitism. In one case, few embryonated eggs were observed and the patient was treated for a possible true parasitism. There is a need to investigate the prevalence of *D. dendriticum* in our country focusing on the distinction between true and spurious infections.

Dicrocoelium dendriticum, known as the lancet liver fluke, is a worldwide-distributed trematode with a complex cycle.¹ The adults are 5–15 mm in length and 1.5–2.5 mm in width and live in the bile ducts of many mammals, particularly Bovidae. *Dicrocoelium dendriticum* needs two intermediate hosts for the complete parasite development. The definitive host (herbivores) is parasitized by eating infected ants, mainly of the genus *Formica*, with metacercariae.² Human infection is quite exceptional and occurs after accidental ingestion of infected ants by eating unwashed raw vegetables or drinking contaminated water. Although the infection is usually asymptomatic, it can produce chronic cholangitis and dilatation of the bile ducts causing weight loss and fatigue.³ However, the most human frequent form is spurious infection after ingesting raw liver of infected animals with eggs or adult worms.⁴

Diagnosis of this parasitism is based on observation of eggs of *D. dendriticum* in feces. Eggs are oval, asymmetrical, and operculated about 35–50 µm size with a brownish thick shell. Eggs in transit (the most common form found) have a variable color shell (yellow, orange, or light brown) with an indistinct dark yellow oval mass, often with 1–4 shiny globules. Embryonated eggs present a uniform dark brown shell and contain a ciliated embryo inside.⁵ The presence of embryonated eggs (true infection) or transit eggs (spurious infection) in the coproparasitological examination would help to identify the type of infection.

Differentiation between true and spurious infection is also carried out by repeating the parasitological study in feces after a diet free of liver or derivatives for at least 3 days. Failure to observe the eggs would indicate false parasitism, whereas observing eggs would indicate a true infection.

Treatment in true parasitism is praziquantel 15 or 25 mg/kg in a single dose or two doses of 7.5 mg on successive days or

single dose of triclabendazole 10 mg/kg. In case of spurious infection, treatment is not recommended.⁶

We present six cases of *D. dendriticum* detected at a Reference International Health Unit located in an urban area with a high level of immigration between April 2015 and January 2016.

The study was carried out at the Microbiology Laboratory located at the Drassanes Tropical Medicine Unit of Vall d'Hebron University Hospital (HUVH) (PROSICS, Barcelona). The period of time was from April 2015 (first case reported) to January 2016. In all patients, an immigrant screening protocol for parasitic diseases (feces, blood, and urine) and a basic biochemical and hematological profiles were performed.

The parasitological study was carried out after the concentration of feces (modified Ritchie method) and subsequent visualization with an optical microscope.⁷

To determine whether it was a true or spurious infection, parasitological study was repeated after indicating the patient a diet free of meat and viscera for at least 3 days prior to a new examination of stool sample.

Epidemiological and clinical data were collected through an epidemiological survey, when possible, and the patient's clinical history review. All data were stored anonymously.

During the period of study, eggs of *D. dendriticum* were visualized in stool samples of six patients from sub-Saharan Africa (three were from Nigeria, two from Senegal, and one from Mali). The mean age was 35.8 years (range 25–52) with an average time of residence in Spain of 10.25 years (range 0.5–24). Of these, four were women and two men. Epidemiological data are given in Table 1.

At the time of the visit, only one patient presented fever and arthralgia and the others were asymptomatic. In one case (case 1), few embryonated and no transit eggs of *D. dendriticum* were found (Figure 1A). This patient, who also had elevated levels of gamma-glutamyl transpeptidase (GGT), was considered truly infected and was treated with praziquantel. In the remaining patients, only transit eggs were observed (Figure 1B). Eggs of *Schistosoma mansoni* were visualized in feces of the other case with a slight

*Address correspondence to Zaira Moure, Department of Microbiology, Vall d'Hebron University Hospital, P. de la Vall d'Hebron 119-129, 08035 Barcelona, Spain. E-mail: zmoure@vhebron.net

TABLE 1
Epidemiological data of the studied cases

Case	Country of origin	Time from arrival to Spain	Residence in Spain	Eating habits	Meat origin
1	Nigeria	8 years	Homeless	Unknown	Unknown
2	Nigeria	1 year	Santa Coloma de Gramenet	Cattle's liver	No veterinary control meat
3	Mali	12 years	Unknown	Unknown	Unknown
4	Senegal	24 years	Granollers	Lamb liver	No veterinary control meat
5	Senegal	16 years	Granollers	Lamb liver	No veterinary control meat
6	Nigeria	6 months	Barcelona	Lamb liver	Controlled regular supermarket

eosinophilia, and the patient was also treated with praziquantel. In addition, four out of the six patients had elevated IgE levels. A second parasitological examination was repeated for all individuals at 4–8 weeks and no *D. dendriticum* eggs were observed in any sample. Table 2 summarizes clinical data of the cases.

There are few human prevalence studies on *D. dendriticum* infection as it is poorly known and often underestimated. In a hospital in Saudi Arabia, eggs were found in the stools of 208 patients over a 3-year period.⁸ Prevalence studies carried out in schools in Kyrgyzstan and Ghana found *D. dendriticum* in 8.0% and 1.91% of children, respectively.^{9,10} In 2007, microcoeliasis was included in the list of causative agents for which burden of disease estimates should be derived by Task Force 1 of the Foodborne Disease Burden Epidemiology Reference Group of the World Health Organization.¹¹

As said before, the differential diagnosis between true and spurious infections should be performed through a thorough examination of stool after a strict diet free of liver or derivatives.² The fact that all our patients belonged to a community that often consumes livestock viscera, and the subsequent no visualizations of *D. dendriticum* after diet, suggests that at least the four untreated cases in our study presented false parasitism. It would be interesting to collect dietary habit information in the immigrant population at the clinics of international health units to help in the management of these parasite infections.

Microscopy can also help in the differentiation between transit and embryonated eggs. In five of our cases, transit eggs were visualized indicating a spurious infection. Few embryonated eggs were observed in feces of only one patient

who was treated with praziquantel for being a possible true parasitism. However, due to lack of follow-up, no further studies could be performed in this patient to confirm the infection. It should be considered that microscopic differentiation requires specialized and experienced human resources in parasitological microscopic diagnosis, which is not always possible, contributing to the underestimation of cases.

None of the patients showed symptoms of true parasitism such as constipation, chronic diarrhea, abdominal pain, or hepatomegaly.¹² Only one patient presented fever and arthralgia probably due to other causes not related to this parasite infection.

Regarding analytical abnormalities, it is noteworthy that four patients had elevated IgE. These high levels of IgE could be explained by other causes (possible concomitant parasitism), given the origin of the patients. One of them also presented eosinophilia because of the concomitant parasitization with *S. mansoni*. The patient in whom embryonated eggs were observed also had elevated values of GGT, reinforcing the suspicion of being a true parasitization.

One of the factors influencing the number of isolated cases of *D. dendriticum* in this short period of time is the characteristics of the population attended in this Tropical Unit: migrants that eat liver as a cultural eating habit, some of them sacrificing the animal at home with no veterinary control. Regarding the prevalence rates of this fluke parasitization in herbivore animals in our country, there are some studies that report variable rates ranging between 23% and 100%.^{2,4,13–15} However, there is still little epidemiological data, making it difficult to know the real situation of this parasitism and to establish control guidelines to combat it.

FIGURE 1. (A) Embryonated egg of *Dicrocoelium dendriticum* containing miracidium from patient 1. (B) Transit egg of *D. dendriticum* from one of the remaining patients.

TABLE 2
Clinical data of the studied cases

Case	Analytical abnormalities	Coproparasitological. First examination	Eggs morphology	Treatment	Coproparasitological. Post-diet examination
1	↑ IgE ↑ GGT	<i>Dicrocoelium dendriticum</i>	Embryonated	Praziquantel	Negative
2	↑ IgE	<i>D. dendriticum</i>	Transit	No	<i>Endolimax nana</i> , <i>Entamoeba dispar</i>
3	Eosinophilia ↑ IgE	<i>Schistosoma mansoni</i> , <i>D. dendriticum</i>	Transit	Praziquantel	<i>Blastoscystis hominis</i>
4	No	<i>D. dendriticum</i>	Transit	No	Negative
5	No	<i>D. dendriticum</i>	Transit	No	Negative
6	↑ IgE	<i>D. dendriticum</i>	Transit	No	Negative

GGT = gamma-glutamyl transpeptidase.

Our findings could be important for public health because of the increased relevance of uncontrolled markets, the traditional food preparation practices among the immigrant population, and the lack of veterinary control guidelines assigning potential sources of this trematode infection.

We consider that it would be very interesting to know the prevalence of the *D. dendriticum*, in our population, both native and immigrant, since it is probably underestimated. Reference laboratories in Tropical Medicine and International Health should also be aware of this issue to support other laboratories with less experience in microscopic diagnosis to provide a correct care in those possible cases.

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Authors' addresses: Zaira Moure, Francesc Zarzuela, Mateu Espasa, Tomas Pumarola, and Elena Sulleiro, Department of Microbiology, Vall d'Hebron University Hospital (HUVH), Universitat Autònoma de Barcelona, PROSICS, Barcelona, Spain, E-mails: zaira_moure@hotmail.com, francesc.bcn.ics@gencat.cat, mespasa@vhebron.net, tpumarola@vhebron.net, and esulleir@vhebron.net. Diana Pou, Nuria Serre-Delcor, Begoña Treviño, Cristina Bocanegra, and Israel Molina, Department of Infectious Diseases, Tropical Medicine Unit Vall d'Hebron-Drassanes, Universitat Autònoma de Barcelona, PROSICS, Barcelona, Spain, E-mails: dpou.bcn.ics@gencat.cat, nserre.bcn.ics@gencat.cat, btrevino.bcn.ics@gencat.cat, cristinabocanegra@gmail.com, and imolina@vhebron.net.

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