

## Morphological and Molecular Detection of *Dicrocoelium Dendriticum*

### Infection in Ruminants

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#### Abstract

**Background:** Dicrocoeliosis is a parasite disease that affects the liver of domestic and wild ruminants. It is one of the difficulties associated with livestock development that causes significant diseases and economic losses to animals.

**Objective:** This study aimed to identify *Dicrocoelium dendriticum* in (sheep,cattle) using their morphological and molecular characteristics

**Methods:** The current study included the examination of 122 livers from both sexes of sheep and cattle that were slaughtered in butcher's shops, worms selected randomly from

20 the livers sheep and cattle stained for parasite morphometric measurements studies , in addition to macroscopically examination of liver depending on the color, consistency and size. Molecular methods are used to confirm the diagnosis of *Dicrocoelium dendriticum* in cattle and sheep.

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25 **Results:** The infection rate of *Dicrocoelium dendriticum* parasite in sheep and cattle were 34.4% ± 16.758, 10.7%± 16.758 , respectively. The results showed a significant difference of the rate of infection between sheep and cattle, While there is no significant difference between females and males in both sheep and cattle. Macroscopic diagnosis of the livers infected showed discoloration, swelling, wrinkled needle- spots shape and hyaline, the sharpness of liver margins loss and biliary fibrosis. all specimens collected from the sheep  
30 and cattle were morphologically identifies as *Dicrocoelium dendriticum*,, .The molecular examination showed the diagnosis of these parasite, which belong to the species *Dicrocoelium dendriticum*, with a product reaction of 900 base pairs.

**Conclusion:** This study is the first isolation and detection of *Dicrocoelium dendriticum*, in the sheep and cattle and to overview the genotype compositions of *Dicrocoelium dendriticum*

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35 **Keywords:** *Dicrocoelium dendriticum*, Dicrocoeliosis in sheep, Dicrocoeliosis in cattle,  
lancet liver fluke, PCR of *Dicrocoelium*

## Introduction

40 *Dicrocoelium dendriticum* called lancet liver fluke is common parasitic diseases of  
liver and gall bladder in wild and domesticated animals, also observed in horses, camel,  
rabbits, pig's and human (Scala *et al.*, 2019). In addition, human infection rate has  
increased in recent years (Rad *et al.*,2022; Abdollahpour *et al.*,2023). *Dicrocoelium*  
*dendriticum* considers as important species in economic industries and veterinary Medicine  
45 (Arbabi *et al.*, 2018). *Dicrocoelium* has a complicated life cycle (Oyibo *et al.*, 2018;  
Hosseini *et al.*.,2022). Its first intermediate hosts are various species of snails, and its  
second intermediate hosts are various species of ants, which able to cause diarrhea, weight  
loss, anemia , digestive disorders, edema, growth delay, reduced milk production, biliary  
obstruction cholangitis acute urticarial, and cirrhosis ( Ofori *et al.*, 2015). Dicrocoeliosis as  
food borne zoonotic diseases caused by three species of *Dicrocoelium*, which include  
50 *Dicrocoelium hospes* and *Dicrocoelium chinensis* (Khan *et al.*,2023). *Dicrocoelium hospes*  
is endemic disease in west Africa while *Dicrocoelium chinensis* in Europe and eastern  
Asia, whereas *Dicrocoelium dendriticum* endemic in Asia, Europe, North Africa, and

America (Chougar *et al.*,2019; Lianou *et al.* ,2023). The purpose of this study was to characterize the morphology and molecular characteristics of the parasite *Dicrocoelium dendriticum* in cattle and sheep as well as to detect its various species in these animals.

## **Material and Methods**

### **Ethical approval**

Ethical approval was issued by the Institutional Animal Care and Use Committee (UM.VET.2022.16) at the College of Veterinary Medicine, University of Mosul on September 6, 2022.

### **Samples Collection**

Total of 122 livers samples taken from 64 sheep and 58 cattle slaughtered in the different areas in Mosul city for both sexes. Worms were placed in 70% ethanol alcohol and stored in- 20 °C for polymerase chain reaction technique (Mahdee *et al.*, 2022).

### **Morphological examination**

Fifteen (50) adult worms were selected randomly from the livers of sheep and cattle, the worm is placed between two slides of glass, and treated with formalin acetic acid alcohol solution, then stained with hematoxylin and mounted in Canada balsam (Ebrahim *et al.*,

70 2020) . Morphometric measurements of parasites were performed under the light and stereo  
of microscope according to (Jwher *et al.*, 2021). Macroscopic examination of liver  
depending on the color, consistency, and size (Makawi *et al.*.,2023; Mahdee *et al.*,2022).

### **DNA Extraction**

75 DNA of 20 worms was extracted using Pure Column DNA Extraction Kit, Bioron, and  
the extraction was attempt according to manufacture manual. Restriction fragment length  
polymorphism (RFLP)-PCR to detect the DNA of *Dicrocoelium dendriticum*, worms,  
REL-PCR was attempt, the amplification of DNA was done using specific primer to  
*Dicrocoelium dendriticum*, NADH dehydrogenase subunit gene (*nad1*) , the primer  
80 Forward (5'TTAAAACACACCCACCAGAA-3'), and primer Reverse (5'-  
GTGTTTTGGTTTAATGTTTT-3')( Gorjipoor *et al.*, 2015). the reaction solution 25 µl  
contain 5 µl of DNA extracted later, 1 µl of each primer, and 12 µl of mater mix  
(IDAA™ – kit) and 6 µl of distilled water. The amplification program include 95°C in 5  
minutes with 30 cycles in 95°C then 2 minutes, 58.4°C in 1 minute and 72°C for 2 minutes.  
85 Final amplification products were diagnosed using 1% agarose gel electrophoresis (900  
bp), after which 2ul of Thermo Scientific™ Fast Digest Green Buffer (10x) (Thermo  
Fisher Scientific Inc.) was added to 10 µl of PCR mix with 18ul of distilled water.

Afterwards it was incubated for 40 minutes at 37 °C. The reaction products (206 and 694 bp) were analyzed by 1% agarose gel electrophoresis (Shokouhi et al., 2018).

## 90 **Statistical Analysis**

Statistical analysis carried out used Chi-square test by SPSS (Version 17; SPSS Inc., Chicago, USA).

## **RESULTS**

95 The infection rate of *Dicrocoelium dendriticum* was 23%, the rate of infection in sheep and cattle were  $34.4\% \pm 16.758$ ,  $10.7\% \pm 16.758$  respectively. The results show a significant difference of infection between both sheep and cattle, The results are not significant at  $P < 0.05$  between males and females for both sheep and cattle (Table 1). Morphometric measurements of *Dicrocoelium dendriticum* in sheep and cattle are given  
100 "in the Table 2", also show the mean of the internal external diameter of the oral and ventral suckers, in addition to diameters of testes, vitelline glands (length, width) and the testes orientation in the *Dicrocoelium dendriticum*. (Tandem testes) "in the Figure 1,2". Current study clarifies that *Dicrocoelium dendriticum* is only species, which isolated from both sheep and cattle according to morphometric study and molecular study. Macroscopic  
105 diagnosis of the infected livers shows discoloration, swelling, wrinkled spots and hyaline, and biliary fibrosis lack of the sharpness of liver margins "in the Figure 3". In addition,

large numbers of flukes detected in the bile ducts of the liver. As for results of molecular analysis, the amplification of DNA of worms by using PCR-RFLP used specific primer of *Dicrocoelium dendriticum* nad1 fragment (900 bp), and then digestion to produce 206 and 694 bp fragments. All samples were positive to *Dicrocoelium dendriticum*, " Figure 4".

## DISCUSSION

Dicrocoeliosis is one of parasitic diseases important in veterinary medicine and economic industries affect the liver of domestic and wild animals (Kleiman *et al.*, 2007). According to the curret study, the percentage of infected sheep and cattle that were slaughtered was 34.4% and 10.7%, respectively, with significant differences between them, This results agreement with the previous study from other country by Ebrahim *et al.* (2020) which declared that the percentage of infection in sheep and cattle were 36.7 %, 6.09 % respectively. While Ahmadi and Mahdavi, (2010) showed that, the percentage of infection in both sheep and cattle were 22.4 %, 4.5%. The differences of the percentage of *Dicrocoelium dendriticum* in Iraq is probably due to part of the grazing behavior, environmental conditions and management system in the country. In addition, the current study evaluated the influence of sex for the host on *Dicrocoelium dendriticum*. Whereas,

no statistical significant effects were observed for the sex in the percentage of  
125 *Dicrocoelium dendriticum* infection (Maurelli *et al.*, 2007).

All specimens collected from the sheep and cattle were morphologically identified  
*Dicrocoelium dendriticum* depending on measurement and morphological keys such as  
body length, maximum body width, testes orientation, and other morphometric  
measurements and this result was agreement with (Sandoval *et al.*, 2013). In addition, the  
130 other studies in Austrian, Italian and German showed the location of testes in *Dicrocoelium*  
*dendriticum* determent the species of *Dicrocoelium*. The testicles of *Dicrocoelium*  
*dendriticum* are tandem, but in *Dicrocoelium chinensis* are bilateral (Javanmard *et*  
*al.*,2022). This is agreement with our current study. The results of macroscopic observation  
of infected livers were in line with the results described by Liu *et al.* (2014); Manga *et al.*  
135 (2010) showed discoloration, swelling, hyaline, change of liver margins and biliary  
fibrosis. . The results of the molecular techniques of this current study are in agreement  
with the results described by Gorjipoor *et al.* (2015). However, according to morphometric  
measurements and molecular techniques, *Dicrocoelium dendriticum* exclusively causes  
diploidosis in sheep and cattle in Mosul, Iraq.

140 **CONCLUSION**



145 This study is regarded as the first to identify *Dicrocoelium dendriticum* in Iraq's Mosul City . The information gathered from the current study's data will reveal the percentage of *Dicrocoelium dendriticum* in Iraq. Moreover, these *Dicrocoelium dendriticum* must be molecularly identified in order to be controlled and prevented.

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**Table 1.** The infection rate of *Dicrocoelium dendriticum*, among slaughtered sheep and cattle in Mosul city.

Animal Type	No. examined animals		No. infected animals		The infection rate ± S.D %		
	male	female	male	female	male	female	Total
Sheep	30	34	9	13	30 <sup>a</sup>	38.2 <sup>a</sup>	34.4*±16.758
Cattle	25	33	2	4	8 <sup>b</sup>	12.1 <sup>b</sup>	10.7*±16.758
Total	55	67	11	17	20	25.4	23

*P*<0.05)( between sheep and cattle at Significant differences\*

<sup>a</sup> No significant difference between females and males in sheep

<sup>b</sup> No significant difference between females and males in cattle

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**Table 2.** Morphometric measurements of *Dicrocoelium dendriticum*, the characteristics of 50 flukes from (25 flukes from sheep and 25 flukes from cattle)

Variables	Sheep	Cattle
	Min-Max(mean $\pm$ S.D)	Min-Max(mean $\pm$ S.D)
Length (body)	4.10-5.10 mm (4.80 $\pm$ 1.40)	7.02-9.01 mm (5.20 $\pm$ 1.50)
Width (body)	1.05-1.60 mm (2.27 $\pm$ 1.12)	1.20-2.50 mm (160.20 $\pm$ 31.50)
The internal diameter of the oral sucker	170-188 $\mu$ m (119.50 $\pm$ 20.6)	120-300 $\mu$ m (160.20 $\pm$ 31.50)
The external diameter of the oral sucker	298-325 $\mu$ m (216.21 $\pm$ 12.3)	300-400 $\mu$ m (230.20 $\pm$ 31.45)
The internal diameter of the ventral sucker	200-260 $\mu$ m (216.21 $\pm$ 12.3)	120-220 $\mu$ m (130 $\pm$ 25.10)
The external diameter of the ventral sucker	300-460 $\mu$ m	300-550 $\mu$ m



	(332 ± 21.65)	(250.11 ± 42.10)
Length of testicle	490-700µm	460-850µm
	(451 ± 44.8)	(650.10 ± 78.15)
Width of testicle	340-500µm	500-700µm
	(500 40.33)	(600 70.61)
Length of vitelline gland	1.10-2.2 mm	1.25-3.20 mm
	(1.80 1.23)	(2.52 1.02)

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290 Figure 1. Severe infection with *Dicrocoelium Dendriticum*, worms isolated from the bile ducts of sheep's liver

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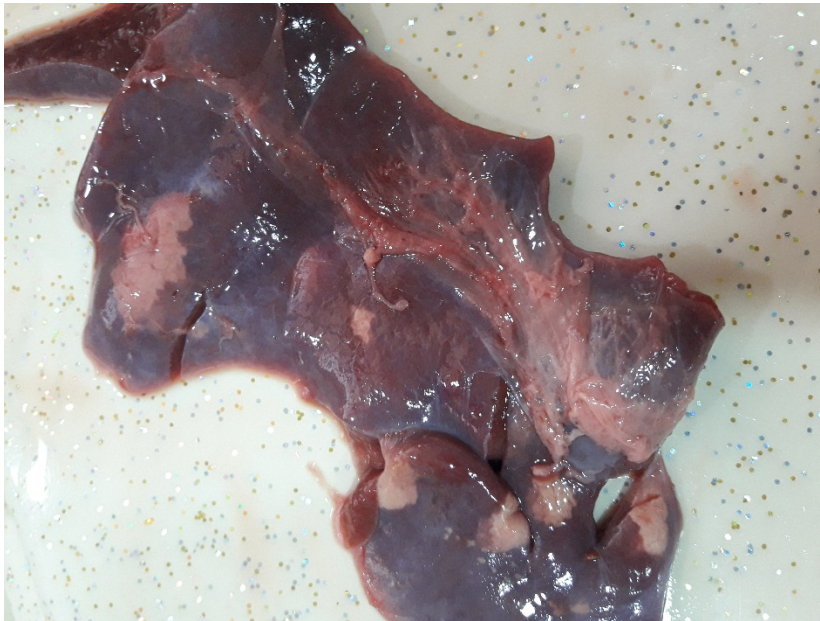


**Figure 2.** *Dicrocoelium dendriticum*, under the dissecting microscope

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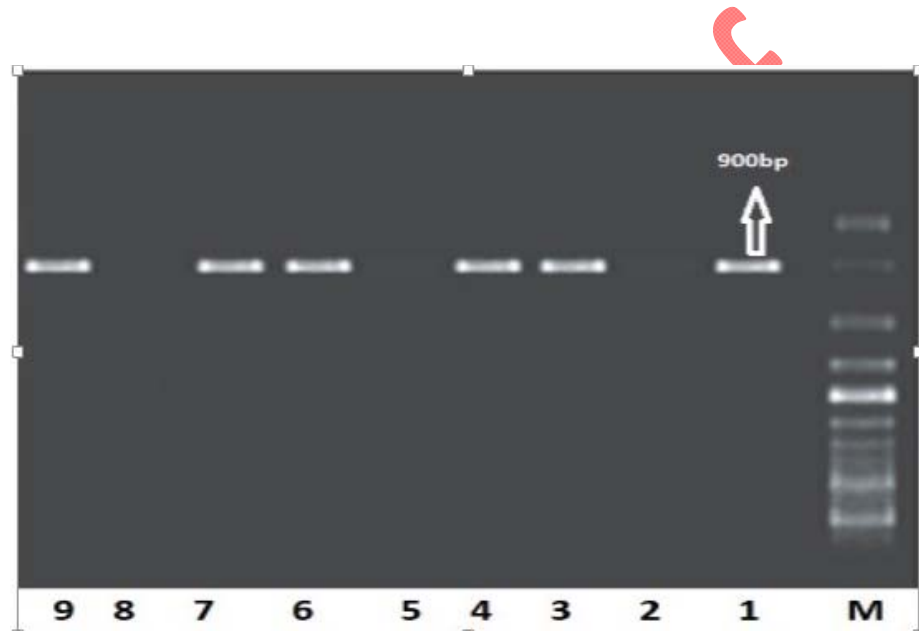
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Figure 3. Livers infected showed discoloration, swelling, hyaline, the sharpness of liver margins loss and biliary fibrosis

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**Figure 4.** Gel electrophoresis image showing: Lane (M) Lanes (2, 5, 8) negative samples to *Dicrocoelium dendriticum* DNA; Lanes (1, 3-4, 6-7 and 9) positive samples to *Dicrocoelium dendriticum* DNA in approximately band size 900 bp.