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(RESEARCH ARTICLE)

PREVALENCE AND EPIDEMIOLOGICAL FACTORS OF *DICROCOELIUM* SPP IN CATTLE AT TAMBOUL SLAUGHTERHOUSE, GEZIRA STATE, SUDAN USING FLOTATION AND SEDIMENTATION TECHNIQUES

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ABSTRACT

Background: *Dicrocoelium dendriticum* and related species are lancet liver flukes that infect the bile ducts of ruminants. *Dicrocoeliosis* leads to cholangitis, liver fibrosis, liver condemnation, and significant economic losses in endemic areas. The life cycle involves terrestrial snails and ants as intermediate hosts, making its distribution highly dependent on environmental and ecological factors.

Objective: To determine the prevalence of _Dicrocoelium_ spp. eggs in slaughtered cattle at Tamboul Slaughterhouse, Gezira State, Sudan, and compare the diagnostic sensitivity of flotation and sedimentation techniques.

Materials and Methods: A cross-sectional abattoir study was conducted from July to August 2022. Three hundred fresh rectal fecal samples were collected. Samples were processed using saturated NaCl flotation and water sedimentation. Eggs were identified morphologically. Data were analyzed using SPSS v26 with Chi-square test.

Results: The overall prevalence was 1.0% (3/300). Sedimentation detected 100% (3/3) of positive cases while flotation detected 66.7% (2/3). Infection was detected only in cattle >3 years 1.7% (3/180). Differences between sexes and breeds were not statistically significant P>0.05.

Conclusion: *Dicrocoelium* infection is of very low prevalence. Sedimentation technique showed higher sensitivity for detection of *Dicrocoelium* eggs. Continuous surveillance is recommended.

Keywords: *Dicrocoelium* spp., Flotation, Sedimentation, Cattle, Prevalence, Gezira State, Sudan.

1 INTRODUCTION

Dicrocoeliosis is a parasitic disease of domestic and wild ruminants caused by trematodes of the genus *Dicrocoelium*, with D. *dendriticum* being the most common species worldwide. Adult flukes reside in the bile ducts and gall bladder, inducing chronic cholangitis, fibrosis, and cirrhosis, which often results in partial or total condemnation of livers at slaughter. The economic impact includes direct losses from condemned livers and indirect losses from reduced weight gain and milk production (Urquhart et al., 2007; Mehlhorn, 2016; Gracey et al., 2019; Radostits et al., 2017; Torgerson and Budke, 2018; Zajac and Conboy, 2012).

The life cycle of *Dicrocoelium* is complex and indirect, requiring two obligate intermediate hosts: a terrestrial snail as first intermediate host and an ant as second intermediate host. Metacercariae are transmitted to definitive hosts when infected ants are ingested during grazing. This dual host dependency restricts the parasite to areas with specific ecological conditions, particularly temperate, semi-arid, and hilly regions where snail and ant populations thrive. Climate, humidity, and vegetation cover are therefore key determinants of transmission (FAO, 2016; Mehlhorn, 2016; Mas-Coma et al., 2009; Hansen and Perry, 2014).

Globally, *dicrocoeliosis* has been reported from Europe, Asia, Africa, and the Americas, with prevalence ranging from <1% to >40% depending on climate and management systems. In Europe and parts of Asia, prevalence can exceed 30% in sheep and goats. In Africa, the disease is sporadic and often underdiagnosed due to low pathogenicity compared to *Fasciola* spp. and lack of routine surveillance (Varcasia et al., 2019; Phiri et al., 2018; Kaufmann, 2016; Ozdal et al., 2015; Ahmed, 2020; Magambo et al., 2016).

In Sudan, irrigated schemes like Gezira State provide favorable conditions for _Fasciola_ but may be unsuitable for *Dicrocoelium* due to lack of suitable snail and ant vectors. The Gezira Scheme is characterized by flat irrigated plains and high temperatures, which are not optimal for most terrestrial snail species involved in *Dicrocoelium* transmission. Tamboul Slaughterhouse receives cattle from diverse agro-ecological zones in Gezira, making it an important sentinel site for monitoring trematode infections (Ahmed, 2020; Mas-Coma et al., 2009).

Diagnosis of dicrocoeliosis relies on detection of characteristic operculated, dark brown eggs 38-45 µm in feces using coprological methods such as flotation and sedimentation. Sedimentation is considered the gold standard for heavy trematode eggs due to their high specific gravity, while flotation may miss some eggs. Despite its economic importance, data on *Dicrocoelium* in Sudanese cattle are scarce. Therefore, this study aimed to determine the prevalence and compare the sensitivity of flotation and sedimentation techniques for detection of *Dicrocoelium* spp. eggs in cattle at Tamboul Slaughterhouse (WHO, 1994; Thienpont et al., 2003; Ahmed, 2020).

2 MATERIALS AND METHODS

2.1 Study Area and Duration

The study was conducted at Tamboul Slaughterhouse, Gezira State, central Sudan 14°16'N 33°32'E. The area has a semi-arid climate with irrigated agriculture and average temperatures of 25-40°C. Samples were collected over 2 months from July to August 2022.

2.2 Study Design and Sampling

A cross-sectional study was designed. A total of 300 cattle were randomly selected at slaughter. Sample size was calculated based on an expected prevalence of 5% with 95% confidence interval and 5% precision. Fresh fecal samples ~20g were collected directly from the rectum immediately after slaughter to avoid environmental contamination. For each animal, data on age, sex, and breed were recorded. Age was categorized as: <1 year, 1-3 years, >3 years. Breeds were classified as Local Sudanese or Crossbreed (Hansen and Perry, 2014; Thienpont et al., 2003).

2.3 Laboratory Examination

All samples were processed within 6 hours of collection at the Parasitology Laboratory, University of AL-BUTANA. Two coprological techniques were applied to each sample to compare sensitivity as recommended by WHO and OIE (OIE, 2021; WHO, 1994):

2.4 Saturated Salt Flotation Technique

3g of feces was emulsified in 42ml of saturated NaCl solution, specific gravity 1.20. The suspension was filtered through a tea strainer into a 15ml test tube. A coverslip was placed on top and left for 20 minutes. The coverslip was then mounted on a slide and examined under x10 and x40 objectives (Soulsby, 2012; Thienpont et al., 2003).

2.5 Water Sedimentation Technique

3g of feces was mixed with 50ml of tap water, stirred, and filtered. The filtrate was left to sediment for 30 minutes. The supernatant was decanted and the sediment examined under microscope. Sedimentation is particularly effective for heavy trematode eggs like *Dicrocoelium* due to their high specific gravity (Taylor et al., 2016; Thienpont et al., 2003).

2.6 Egg Identification

Dicrocoelium eggs were identified based on morphological criteria: small size 38-45 µm, dark brown color, operculum, and asymmetrical shape. Slides were examined by two independent observers to reduce observer bias (WHO, 1994; Chatterjee, 2017; Thienpont et al., 2003).

2.7 Data Analysis

Data were entered into Microsoft Excel and analyzed using SPSS version 26. Prevalence was calculated as: Number positive / Total examined × 100. Sensitivity of each technique was calculated as: True positives by technique / Total true positives × 100. Association between prevalence and risk factors was tested using Chi-square test. $P<0.05$ was considered statistically significant (Bowman, 2019).

3 RESULTS

Table 1: Overall prevalence of *Dicrocoelium* spp. eggs in cattle at Tamboul Slaughterhouse

Parameter	No. Examined	No. Positive	Prevalence %
Total	300	3	1.0

Table 1 presents the overall prevalence. Out of 300 cattle examined, only 3 were positive, giving an overall prevalence of 1.0%. This very low rate suggests that dicrocoeliosis is not a major parasitic problem in cattle slaughtered at Tamboul during the study period. The result aligns with previous reports from irrigated areas of Sudan where prevalence was <2% (Ahmed, 2020).

Table 2: Comparison of diagnostic sensitivity between flotation and sedimentation techniques

Technique	No. Positive Detected	Total True Positives	Sensitivity %
Sedimentation	3	3	100.0
Flotation	2	3	66.7

positive cases, achieving 100% sensitivity, while flotation detected only 2 cases with 66.7% sensitivity. This indicates that sedimentation is more reliable for detection of *Dicrocoelium* eggs because the eggs are heavy 38-45 µm and operculated, causing them to settle rather than float in saturated NaCl solution. Similar findings were reported by Thienpont et al. (2003) and WHO (1994) who recommended sedimentation as the preferred method for trematode eggs. Therefore, using both techniques in parallel increases detection rate, but sedimentation alone would have been sufficient in this study.

Table 3: Prevalence of *Dicrocoelium* spp. in relation to sex, age and breed

Risk Factor	Category	No. Examined	No. Positive	Prevalence %	P-value
Sex	Male	150	2	1.3	0.56
	Female	150	1	0.7	
Age	<1 year	50	0	0.0	0.12
	1-3 years	70	0	0.0	
	>3 years	180	3	1.7	
Breed	Local	170	2	1.2	0.68
	Crossbreed	130	1	0.8	

Table 3 shows the epidemiological breakdown. All positive cases were restricted to cattle older than 3 years 1.7%. This age pattern reflects cumulative exposure over time and the chronic nature of _Dicrocoelium_ infection. The absence of infection in younger animals <3 years suggests limited grazing exposure or that infection requires prolonged contact with infective stages (Urquhart et al., 2007; Ozdal et al., 2015).

Males showed slightly higher prevalence 1.3% than females 0.7%. This may be due to longer retention of males for fattening and increased grazing time, leading to higher exposure. Local breeds 1.2% had marginally higher rates than crossbreeds 0.8%, possibly due to extensive grazing patterns of local breeds compared to semi-intensive management of crossbreeds (Radostits et al., 2017; Ahmed, 2020).

However, Chi-square analysis revealed no statistically significant association between infection and sex, age or breed $P>0.05$. The lack of significance is likely due to the very low number of positive cases n=3, which limits statistical power (Bowman, 2019).

4 DISCUSSION

The overall prevalence of 1.0% recorded in this study indicates that *Dicrocoelium* infection is of very low endemicity in cattle slaughtered at Tamboul during July-August 2022. This finding is consistent with reports from other irrigated areas of Sudan where prevalence ranged from 0.5% to 2.3%, but contrasts sharply with high prevalence >20-40% reported from temperate highland areas in Europe, Turkey and Italy (Ahmed, 2020; Ozdal et al., 2015; Varcasia et al., 2019).

A key finding of this study is the difference in diagnostic sensitivity between the two techniques. Sedimentation detected 100% of cases while flotation detected only 66.7%. This is because *Dicrocoelium* eggs are heavy and operculated, with specific gravity higher than saturated NaCl solution. Therefore, they tend to sediment rather than float. This result supports recommendations by WHO (1994) and Thienpont et al. (2003) that sedimentation is the method of choice for trematode eggs. Using both methods increased confidence in the results, but for resource-limited laboratories, sedimentation alone would be adequate for *Dicrocoelium* screening.

The low prevalence can be explained by the ecology of *Dicrocoelium*. The parasite requires specific terrestrial snails Zonitoides, Cionella and ants Formica as intermediate hosts. These hosts prefer humid, hilly, and temperate habitats with moderate rainfall rather than the hot, irrigated plains of Gezira. The intensive irrigation, high summer temperatures >40°C, and flat topography in Gezira State may limit snail survival and ant activity, thereby breaking the transmission cycle. Similar ecological explanations have been given for low dicrocoeliosis in other arid and irrigated regions (FAO, 2016; Mehlhorn, 2016; Mas-Coma et al., 2009; Hansen and Perry, 2014; Keyyu et al., 2017; Phiri et al., 2018).

Age-related prevalence showed that all positive cases 1.7% were in cattle >3 years. This reflects cumulative exposure over time and the long prepatent period of the parasite. The absence of infection in young animals <3 years suggests that recent transmission is rare in the study area, which is consistent with the low overall prevalence. Similar age patterns have been reported in Turkey and Zambia, where older animals had significantly higher rates (Urquhart et al., 2007; Ozdal et al., 2015; Phiri et al., 2018).

Males 1.3% had slightly higher prevalence than females 0.7%, and local breeds 1.2% had higher rates than crossbreeds 0.8%. The higher rate in males may be related to management practices where males are kept longer for meat production and thus have prolonged exposure to contaminated pastures. Local breeds may be more exposed due to extensive grazing systems compared to crossbreeds that are often stall-fed. However, these differences were not statistically significant $P>0.05$, likely due to the small number of positives (Radostits et al., 2017; Ahmed, 2020).

Although prevalence is low, even a single infected liver can cause economic loss through condemnation. Furthermore, low prevalence does not rule out sporadic outbreaks if intermediate hosts are introduced through livestock movement or environmental changes. Climate change may also alter the distribution of snails and ants in the future. Therefore, surveillance remains important for early detection (Gracey et al., 2019; Torgerson and Budke, 2018; Mas-Coma et al., 2009).

5 CONCLUSION

This study demonstrated a very low prevalence 1.0% of *Dicrocoelium* spp. eggs in cattle at Tamboul Slaughterhouse, confined to older animals >3 years. Sedimentation technique showed superior sensitivity 100% compared to flotation 66.7% for detection of *Dicrocoelium* eggs. The low rate is likely attributed to ecological unsuitability for intermediate hosts in Gezira State (Mas-Coma et al., 2009; Hansen and Perry, 2014).

RECOMMENDATIONS

Based on these findings, we recommend

- Use sedimentation as primary diagnostic method for *Dicrocoelium* screening in abattoirs and field surveys due to its higher sensitivity (WHO, 1994; Thienpont et al., 2003).
- Continuous surveillance: Routine coprological examination should be maintained to detect any future increase in prevalence, as recommended by OIE (OIE, 2021).
- Ecological studies: Survey the presence and distribution of potential snail and ant intermediate hosts in and around Gezira State to identify risk areas (FAO, 2016).
- Proper carcass disposal: Infected livers should be condemned and safely disposed of by burning or deep burial to prevent egg contamination of pastures (WHO, 2015).
- Farmer education: Educate farmers on the life cycle of liver flukes, risk areas, and avoidance of grazing near snail habitats (Gracey et al., 2019; Radostits et al., 2017; Smyth, 2015).
- Further research: Molecular studies using PCR are needed to confirm species and assess genetic diversity of *Dicrocoelium* in Sudan (Mehlhorn, 2016; Smyth, 2015; Mehlhorn, 2016).

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Availability of data and materials

Available from corresponding author on reasonable request. Is our original work and has not been previously published, nor is it under consideration for publication elsewhere.

Author contribution

- All authors have made a substantial contribution to the conception, design, execution, or interpretation of the research.
- We confirm that the data and results presented herein are accurate and were derived and analyzed with full scientific credibility and integrity.

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