



# Prevalence of Bovine Trematodiasis and Associated Risk Factors in Nyagatare District, Rwanda

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**Introduction:** Trematodiasis cause significant financial losses to livestock worldwide and some of which are zoonotic, raising public health concerns. In Rwanda, information on the prevalence of bovine trematodiasis is scanty, and this hampers efforts to control and prevent them in the country.

**Methods:** This cross-sectional study aimed to determine the prevalence of bovine trematodiasis in Nyagatare district and associated risk factors. One hundred cattle were randomly selected for this study and faecal samples were collected directly from the rectum to identify trematode eggs using a simple sedimentation technique. To analyze the data, frequencies, chi-square test, and binary logistic regression were computed.

**Results:** Overall, the prevalence of bovine trematodiasis was 69%, and *Paramphistomum* spp. predominated (69%), followed by *Dicrocoelium* spp. (23%), *Fasciola* spp. (20%), and *Echinostoma* spp. (1.0%). The study also recorded mixed paramphistomiasis, fascioliasis and dicrocoeliasis (11.6%), paramphistomiasis and fascioliasis (15.9%) as well as paramphistomiasis and dicrocoeliasis (20.3%). The odds of having trematodiasis (mono or mixed fascioliasis and dicrocoeliasis) for the cow located in Barija cell (AOR = 0.143; 95% C.I. 0.026–0.793) were 14% lower compared to those of developing such parasitosis for the cow located in Bushoga cell.

**Conclusion:** Taken together, the study shows that trematodes are a significant contributor to lowering livestock production and productivity and pose a threat to human health. Different approaches should be applied to prevent and control the trematodiasis in cows and other livestock (sheep and goats) and reduce the risk of contracting fascioliasis and echinostomiasis in humans in Nyagatare district, Rwanda.

**Keywords:** prevalence, cattle, paramphistomiasis, fascioliasis, echinostomiasis, dicrocoeliasis, Nyagatare district, Rwanda

## Introduction

Agriculture is a vital sector in Rwanda's journey towards becoming a middle-income economy.<sup>1</sup> The sector is critical in achieving long-term economic growth and improving citizen livelihoods. Approximately two-thirds of the working population is employed in agriculture<sup>2</sup> which contributes around 30% of the GDP and employs over 70% of the Rwandan population.<sup>3</sup> The livestock subsector output is a major component of agriculture and contributes 3% of the overall agriculture GDP.<sup>3</sup> Cattle production system in Rwanda is a mixed farming system characterized majorly by keeping Ankole-Friesian crosses that graze on natural communal pastures.<sup>4</sup> In Rwanda, cattle contribute to livelihoods as they supply milk, meat, manure, and cash to the communities and at the same time, they are also used in social and cultural necessities. Worldwide, cattle are a major contributor to animal protein, supplying a good percentage of daily meat.<sup>5</sup> In Rwanda, the indigenous long-horned Ankole cattle and exotic breeds, particularly the Holstein Friesian, are the most common breed raised – Ankole cattle represent 76% of the national cattle herd.<sup>5</sup>

On the other hand, Mazimpaka et al,<sup>4</sup> reported that gastrointestinal parasitoses hold back livestock production in the country. Indeed, these parasites cause tremendous economic losses through reduced milk and meat productivity,

fertility, work capacity, food intake, weight gain, treatment costs, involuntary culling, and death of heavily infected cattle.<sup>6–8</sup> Gastrointestinal trematodes (*Fasciola* spp, *Dicrocoelium* spp, *Paramphistomum* spp) cause serious economic losses globally and are zoonotic (*Fasciola* spp) and thus of public health concern.<sup>9–11</sup> For example, a microscopy-based study carried out in Tanzania reported a prevalence of human fascioliasis accounting for 21%.<sup>12</sup> The losses are due to reduced productivity and damages done to the animal organ, for instance, the liver parasitized with the trematodes is condemned at slaughter.<sup>7,13</sup> Snails are the common intermediate hosts of most trematodes, therefore their occurrence and how definitive hosts are grazed determine the epidemiology and seasonal pattern of trematodiasis.<sup>10,14,15</sup> Trematodiasis' incidences occur in immense water-lodged, marshy grazing fields and this supports their propagation and continuation in the snail intermediate hosts and therefore high prevalence of infection.<sup>16</sup> Important genera of flukes recorded in ruminants in different places include *Fasciola*, *Dicrocoelium*, *Paramphistomum*, and *Schistosoma*.<sup>9,10,17</sup> Trematodiasis that significantly hinder livestock production profitability worldwide, particularly in the humid tropics and sub-tropics, are fascioliasis, paramphistomiasis, and schistosomiasis.<sup>15,18</sup> The prevalence of helminthiasis and their severity vary significantly depending on the climatic conditions of the area, the type of vegetation, and management practices.<sup>6,15,19</sup>

In 2019, the prevalence of bovine helminthiasis in Rwanda accounted for 24.5%.<sup>2</sup> In a study that investigated bovine hepatic lesions at an abattoir in the country, fascioliasis topped the condemnations with 78.7%.<sup>20</sup> Again, a copromicroscopic study conducted on Ankole cattle in Nyagatare district in Rwanda reported the prevalence of fascioliasis totaling 19.9% and 49.3% at individual cow level and herd level, respectively.<sup>21</sup> Copromicroscopic studies conducted on cattle in other countries reported the prevalence of trematodiasis that varied between 50% and 68.9%.<sup>7,10,17,18</sup> Therefore, the study investigated bovine trematodiasis prevalence in Nyagatare district, Rwanda to generate data that could assist in designing effective control measures.

## Materials and Methods

### Study Area

The study area was Nyagatare district, which is among the 7 districts of Eastern Province, Northeastern extremity of Rwanda. The district comprises 14 Sectors and is further subdivided into 106 administrative cells. Nyagatare district is bordered in the East by Tanzania, the north by Uganda, the west by Gicumbi District, and Gatsibo District in the south. The total surface area of the district is 1919 Km<sup>2</sup> and it is considered the largest district in Rwanda.

In 2022, the cattle population in Rwanda was 1,424,180 and the Eastern province was the populous region with 390,826 cattle. Again, Nyagatare district was home to 8.6% of the national cattle population, i.e., the populous district with 122,638 cattle.<sup>22</sup> Thus, Nyagatare district was purposively selected in the first instance, as the study district. Nyagatare district is in a region with low altitude and an average rainfall ranging from 1250 to 1500 mm. Its rainfall is less varied with an annual average temperature varying between 25.3°C and 27.70°C. In the second instance, Nyagatare sector was selected. In 2022, Nyagatare district record showed that families that reared cows were 13,355 including 8.7% who lived in Nyagatare sector. The latter is generally made by savannah vegetation and some forest gallery, and it is subdivided in 9 administrative cells. The study sector (Nyagatare) and cells are shown (Figure 1).<sup>23</sup>

### Study Design and Sample Size

This cross-sectional study recruited apparently healthy cows for fecal sampling. Cows were randomly selected and those aged below 18 months were considered young while those aged above 18 months were considered old. Both male and female cows were sampled, and cattle were of local, exotic, and crossbreeds. The sample size was computed based on the cattle population in Nyagatare sector in 2022 (19,734 cows – as per records of the sector administration) and previously reported copromicroscopic prevalence (49.3%).<sup>21</sup> Cochran's formula for calculating sample size for proportions<sup>24</sup> was used to compute the sample size of study cows as follows:



**Figure 1** A map of the study area – shows the study sector (bounded by red limits) and its nine cells. Only study cows situated in Nyagatare, Barija, Bushoga, Rutaraka, and Nsheke cells were sampled.

$$\frac{Z^2 p(1-p)/e^2}{1 + Z^2 \cdot p(1-p)/e^2 N} = \frac{1.96^2 \times 0.493(1 - 0.493)/0.10^2}{1 + 3.8416 \times 0.249951/0.01 \times 19,734} = 95.5 \approx 96 \text{ cows}$$

Where  $N$  is the population;  $e$  is the precision level and  $p$  is the previously reported prevalence. To compensate for possible non-response, we increased the sample size by 10%,<sup>25</sup> i.e., our target sample was 105 cows of which 100 were sampled successfully.

## Collection of Fecal Samples and Analysis

Each fecal sample was directly collected from the rectum using a rectal palpation glove that was turned inside out to serve as a fecal container. The sample was labeled with relevant information (study location, cow's age, sex, and breed). In the field, the samples were kept in a cool box and transported to the parasitology laboratory of the University of Rwanda, School of Veterinary Medicine for coprological analysis using a simple fecal egg sedimentation technique.<sup>17,26</sup>

For preparing a fecal suspension, 3 grams of feces were suspended in 50 mL of tap water in a beaker and mixed thoroughly.<sup>17</sup> The mixture was then filtered into another beaker using a tea strainer and left undisturbed for 10 minutes. After discarding the supernatant, the sediment was re-suspended three times in 50 mL of water – each time the sediment was left undisturbed for 10 minutes. Finally, the supernatant was discarded carefully and the sediment was poured on a petri dish where a 1% methylene blue drop was added. The dye stained the fecal particles a deep blue leaving the trematode eggs unstained. Finally, the eggs were identified using a light microscope at 10x magnification and based on their morphological characteristics.

## Data Analysis

The data was entered into Excel Microsoft 365 and transferred to an IBM Statistical Package for Social Sciences (SPSS) version 23 for analysis. To calculate the prevalence, the number of study cows infected with the trematode(s) was divided by the total number of study cows and then multiplied by a hundred. Pearson chi-square test of independence<sup>27</sup> and

binary logistic regression were also computed to determine the effect of the cow's location (study administrative cell), age, breed, and sex on the prevalence of trematodiasis. The statistical tests were performed at a precision level of 5%.

## Results

In total, 100 fecal samples were collected – cows were in various administrative cells and were also of different age, sex, and breeds. Information on the overall prevalence of bovine trematodiasis in Nyagatare district is detailed (Table 1).

Bovine trematodiasis prevalence in relation to the study location is detailed (Table 2).

The prevalence of bovine trematodiasis in Nyagatare district in relation to the cow's sex is shown (Table 3).

The prevalence of bovine trematodiasis in Nyagatare district in relation to the cow's age is shown (Table 4).

The prevalence of bovine trematodiasis in Nyagatare district in relation to the cow's breed is detailed in Table 5.

The occurrence of single and mixed bovine trematodiasis in Nyagatare district is itemized in Table 6.

The results of binary logistic regression are presented in Table 7.

**Table 1** Overall Prevalence of Bovine Trematodiasis in Nyagatare District, Rwanda (n = 100)

| Trematodiasis Status | Frequency  | Percent (%) |
|----------------------|------------|-------------|
| Positive             | 69         | 69.0        |
| Negative             | 31         | 31.0        |
| <b>Total</b>         | <b>100</b> | <b>100</b>  |

**Note:** Table 1 reveals that the overall prevalence of bovine trematodiasis in Nyagatare district accounted for 69%.

**Table 2** Prevalence of Bovine Trematodiasis in Nyagatare District According to the Study Location

| Trematode Genus           | Location  | Number of Study Cows | Proportional Percent s (%) | Prevalence (%) | Test of Independence (p-Value) |
|---------------------------|-----------|----------------------|----------------------------|----------------|--------------------------------|
| <i>Paramphistomum</i> spp | Bushoga   | 20                   | 14 (20.3)                  | 70             | 0.087                          |
|                           | Rutaraka  | 20                   | 18 (26.1)                  | 90             |                                |
|                           | Barija    | 20                   | 15 (21.7)                  | 75             |                                |
|                           | Nsheke    | 20                   | 11 (15.9)                  | 55             |                                |
|                           | Nyagatare | 20                   | 11 (15.9)                  | 55             |                                |
| Total                     |           | 100                  | 69 (100)                   | 69             |                                |
| <i>Fasciola</i> spp       | Bushoga   | 20                   | 10(50)                     | 50             | 0.006                          |
|                           | Rutaraka  | 20                   | 3(15)                      | 15             |                                |
|                           | Barija    | 20                   | 2(10)                      | 10             |                                |
|                           | Nsheke    | 20                   | 2(10)                      | 10             |                                |
|                           | Nyagatare | 20                   | 3(15)                      | 15             |                                |
| Total                     |           | 100                  | 20(100)                    | 20             |                                |

(Continued)

**Table 2** (Continued).

| Trematode Genus         | Location  | Number of Study Cows | Proportional Percent s (%) | Prevalence (%) | Test of Independence (p-Value) |
|-------------------------|-----------|----------------------|----------------------------|----------------|--------------------------------|
| <i>Dicrocoelium</i> spp | Bushoga   | 20                   | 2(10)                      | 8.7            | 0.002                          |
|                         | Rutaraka  | 20                   | 0                          | 0              |                                |
|                         | Barija    | 20                   | 10(50)                     | 43.5           |                                |
|                         | Nsheke    | 20                   | 5(25)                      | 21.7           |                                |
|                         | Nyagatare | 20                   | 6(30)                      | 26.1           |                                |
| Total                   |           | 100                  | 23(100)                    | 23             |                                |

**Notes:** Table 2 shows that *Paramphistomum* spp was the predominant trematode (69%), followed by *Dicrocoelium* spp (23%), *Fasciola* spp (20%), and *Echinostoma* spp (1%)([Supplementary Figures S1–S4](#)). The chi-square test showed that the cow's location (study cell) influenced the prevalence of fascioliasis and dicrocoeliasis ( $p < 0.05$ ).

**Table 3** Prevalence of Bovine Trematodiasis in Nyagatare District in Relation to the Cow's Sex

| Trematode                 | Sex    | Number of Study Cows | Proportional Percent (%) | Prevalence (%) | Test of Independence (p-value) |
|---------------------------|--------|----------------------|--------------------------|----------------|--------------------------------|
| <i>Paramphistomum</i> spp | Female | 84                   | 57(67.9)                 | 82.6           | 0.571                          |
|                           | Male   | 16                   | 12(75)                   | 17.4           |                                |
| Total                     |        | 100                  | 69(69)                   | 100            |                                |
| <i>Fasciola</i> spp       | Female | 84                   | 11(13.1)                 | 55             | <0.001                         |
|                           | Male   | 16                   | 9(56.2)                  | 45             |                                |
| Total                     |        | 100                  | 20(20)                   | 100            |                                |
| <i>Dicrocoelium</i> spp   | Female | 84                   | 17(20.2)                 | 73.9           | 0.133                          |
|                           | Male   | 16                   | 6(37.5)                  | 26.1           |                                |
| Total                     |        | 100                  | 23(23)                   | 100            |                                |

**Notes:** The results in [Table 3](#) show that trematodiasis predominated in females which is 82.6%, 55%, and 73.9% for *Paramphistomum* spp, *Fasciola* spp, and *Dicrocoelium* spp respectively. The results show a significant association of fascioliasis with the cow's sex but at the level of 10% ( $p < 0.001$ ).

**Table 4** Prevalence of Bovine Trematodiasis in Nyagatare District in Relation to the Cow's Age

| Trematode                 | Age   | Number of Study Cows | Proportional Percent (%) | Prevalence (%) | Test of Independence (p-value) |
|---------------------------|-------|----------------------|--------------------------|----------------|--------------------------------|
| <i>Paramphistomum</i> spp | Adult | 67                   | 46(68.7)                 | 66.7           | 0.916                          |
|                           | Young | 33                   | 23(69.7)                 | 33.3           |                                |
| Total                     |       | 100                  | 69(69)                   | 100            |                                |
| <i>Fasciola</i> spp       | Adult | 67                   | 5(7.5)                   | 25             | 0.000                          |
|                           | Young | 33                   | 15(45.5)                 | 75             |                                |
| Total                     |       | 100                  | 20(20)                   | 100            |                                |

(Continued)

**Table 4** (Continued).

| Trematode               | Age   | Number of Study Cows | Proportional Percent (%) | Prevalence (%) | Test of Independence (p-value) |
|-------------------------|-------|----------------------|--------------------------|----------------|--------------------------------|
| <i>Dicrocoelium</i> spp | Adult | 67                   | 9(13.4)                  | 39.1           | 0.002                          |
|                         | Young | 33                   | 14(42.4)                 | 60.9           |                                |
| Total                   |       | 100                  | 23(23)                   | 100            |                                |

**Notes:** The results in Table 4 show that paramphistomiasis (66.7%) was predominant in the adult cattle while fascioliasis (75%) and dicrocoeliasis (60.9) predominated in the young cattle. The prevalence of dicrocoeliasis was influenced by the age of the cattle ( $p < 0.05$ ). The age also influenced the occurrence of fascioliasis ( $p < 0.001$ ).

**Table 5** Prevalence of Bovine Trematodiasis in Nyagatare District in Relation to the Cow's Breed

| Trematode                 | Breed  | Number of Study Cows | Proportional Percent (%) | Prevalence (%) | Test of Independence (p value) |
|---------------------------|--------|----------------------|--------------------------|----------------|--------------------------------|
| <i>Paramphistomum</i> spp | Cross  | 94                   | 64(68.1)                 | 92.8           | 0.606                          |
|                           | Exotic | 2                    | 2(100)                   | 2.9            |                                |
|                           | Local  | 4                    | 3(75)                    | 4.3            |                                |
| Total                     |        | 100                  | 69(69)                   | 100            |                                |
| <i>Fasciola</i> spp       | Cross  | 94                   | 16(17.0)                 | 80.0           | 0.005                          |
|                           | Exotic | 2                    | 2(100)                   | 10             |                                |
|                           | Local  | 4                    | 2(50)                    | 10             |                                |
| Total                     |        | 100                  | 20                       | 100            |                                |
| <i>Dicrocoelium</i> spp   | Cross  | 94                   | 19(20.2)                 | 82.6           | 0.025                          |
|                           | Exotic | 2                    | 1(50)                    | 4.3            |                                |
|                           | Local  | 4                    | 3(75)                    | 13             |                                |
| Total                     |        | 100                  | 23                       | 100            |                                |

**Notes:** Table 5 shows that paramphistomiasis (92.8%), fascioliasis (80.0%), and dicrocoeliasis (82.6%) were more prevalent in crossbreeds than in the other two breeds. The occurrence of fascioliasis and dicrocoeliasis was also associated with the cow's breed ( $p < 0.05$ ).

**Table 6** Occurrence of Single and Mixed Bovine Trematodiasis in Nyagatare District (N = 69)

| Trematode Genus   | Frequency of the Infected | Percent |
|---|---------------------------|---------|
| <i>Paramphistomum</i> spp   | 35                        | 50.7    |
| <i>Paramphistomum</i> spp, <i>Fasciola</i> spp, <i>Dicrocoelium</i> spp, and <i>Echinostoma</i> spp | 1                         | 1.4     |
| <i>Paramphistomum</i> spp, <i>Fasciola</i> spp, and <i>Dicrocoelium</i> spp                         | 8                         | 11.6    |
| <i>Paramphistomum</i> spp and <i>Fasciola</i> spp   | 11                        | 15.9    |
| <i>Paramphistomum</i> spp and <i>Dicrocoelium</i> spp   | 14                        | 20.3    |
| Total   | 69                        | 100     |

**Note:** The results in Table 6 show that mixed paramphistomiasis and dicrocoeliasis predominated at 20.3% followed by that of paramphistomiasis and fascioliasis standing at 15.9%.

**Table 7** Effect of the Cow's Study Location, Age, and Breed on the Occurrence of Mono or Mixed Fascioliasis and Dicrocoeliasis in Nyagatare District

| Variables      | Categories | Adjusted Odds Ratio (AOR) | 95% C.I. for AOR |        |
|----------------|------------|---------------------------|------------------|--------|
|                |            |                           | Lower            | Upper  |
| Study location | Bushoga    | 1                         | Reference        |        |
|                | Rutaraka   | 0.538                     | 0.146            | 1.983  |
|                | Barija     | 0.143                     | 0.026            | 0.793  |
|                | Nsheke     | 0.418                     | 0.109            | 1.606  |
|                | Nyagatare  | 1.167                     | 0.322            | 4.234  |
| Age            | <18 months | 1                         | Reference        |        |
|                | >18 months | 1.065                     | 0.399            | 2.839  |
| Breed          | Cross      | 1                         | Reference        |        |
|                | Exotic     | 1.992                     | 0.174            | 22.794 |
|                | Local      | 0.000                     | 0.000            | –      |
| Constant       |            | 0.404                     |                  |        |

**Notes:** Binary logistic regression analysis shows that the age and breed of the cows were not significantly associated with the prevalence of trematodiasis (fascioliasis and dicrocoeliasis) while the cows' location was significantly associated with it. Specifically, the odds of having trematodiasis (mono or mixed fascioliasis and dicrocoeliasis) for the cow located in Barija cell (AOR = 0.143; 95% C.I. 0.026–0.793) were 14% lower compared to those of developing such parasitosis for the cow located in Bushoga cell.

## Discussion

The overall bovine prevalence of trematodiasis in Nyagatare district, Rwanda was 69.0% and the cow's location (study cell) influenced the prevalence of fascioliasis and dicrocoeliasis. This study's overall prevalence was higher than 57%, 50%, 61%, and 52.6% reported in Tanzania, Indonesia, and Ethiopia, respectively.<sup>7,10,17,19</sup> It was, however, comparable to 68.9% reported in Bangladesh<sup>18</sup> and lower than 78.1% recorded in Nigeria.<sup>9</sup> The dissimilarities in prevalence in the different studies might be due to variations in ecological and climatic conditions. The infection rate of pasture is the major epidemiological variable influencing the trematode burden of animals.<sup>16,28,29</sup> Indeed, trematodiasis occurrence depends on the presence of the snails' intermediate host.

This current study recorded *Paramphistomum* spp as the prevalent trematode followed by *Fasciola* spp and *Dicrocoelium* spp, while *Echinostoma* spp was the least prevalent. Similar patterns of occurrence of *Paramphistomum* spp, *Fasciola* spp, and *Dicrocoelium* spp were also reported in Nigeria.<sup>9,16</sup> Similarly, studies done in Tanzania and Ethiopia<sup>10,17</sup> found *Paramphistomum* spp to be predominant followed by *Fasciola* spp. We found that the most prevalent trematode was *Paramphistomum* spp (69.0%) and this was higher than 62.8%, 50.5%, and 47% reported in Tanzania, Bangladesh, and Indonesia, respectively.<sup>7,18,30</sup> It was, however, lower than 76% reported in Zambia.<sup>13</sup> The high prevalence in the current study may be due to the abundance of intermediate hosts in the environment, grazing on infected pastures, contaminated water sources and the lack of efficient helminth management systems in the study area.<sup>14</sup> Furthermore, it may be related to parasite biology, its ability to survive for a long time in the host, and also its capacity to produce many eggs, consequently massive development in the affected snails.<sup>17,29</sup> Additionally, it is reported that *Paramphistomum* spp occur worldwide, can adapt to a wide range of snails<sup>19</sup> and are also common in the tropical and sub-tropical regions.<sup>14,28,29</sup> Furthermore, bovine paramphistomiasis has been reported as an endemic disease in East African countries.<sup>19,31</sup> A study conducted in Zambia<sup>32</sup> reported a higher prevalence of *Fasciola* spp (68.8%) compared to



*Paramphistomum* spp (50.0%) and this might be due to the abundance of *Fasciola* intermediate hosts in Zambia compared to Rwanda (Nyagatare district). A study done in Nigeria also reported a higher prevalence of *Fasciola* spp (74.9%) followed by *Paramphistomum* spp (16.1%), *Dicrocoelium* spp (7.3%), and *Schistosoma* spp (1.2%)<sup>9</sup> and this might be due to high adaptability of *Fasciola* intermediate hosts in the environment of North-central Nigeria.

Contrarily, the low *Fasciola* prevalence recorded in this study was comparable to 20.1% and 22.2% reported in Ethiopia.<sup>17,19</sup> It was however lower than 18% and 19% that were previously reported in the Democratic Republic of Congo and Rwanda, respectively.<sup>21,33</sup> On the other hand, a study done on slaughtered cattle at Nyabugogo abattoir in Rwanda reported a higher prevalence of fasciolosis (78.7%) and cattle from Nyagatare district had a prevalence of 30.2%.<sup>20</sup> Other studies have also recorded a higher prevalence of fascioliasis, 67% and 48% in Malaysia and Indonesia, respectively.<sup>7,34</sup> It is reported that the risk of fasciolosis happens in areas with higher annual rainfall and with the risk-reducing in areas with shorter wet seasons and/or lower temperatures, since in hot conditions metacercariae survival is reduced<sup>16,35</sup> like what is experienced in the current study area.

Differently, the prevalences of *Dicrocoelium* spp reported in other studies were 7.3%,<sup>9</sup> 5.68%,<sup>36</sup> and 0.52%<sup>37</sup> which were lower than that reported in this study (23.0%), and this might be due to the presence of ants as second intermediate hosts for the parasite in Nyagatare district and this factor could contribute to the higher prevalence compared to other regions. However, a higher prevalence of *Dicrocoelium* spp has been reported elsewhere, i.e., 37.6% in Spain<sup>38</sup> and 39% in Nigeria.<sup>39</sup> In addition, the survivability and distribution of *Dicrocoelium* spp is reported to be sustained by environmental factors, intermediate host, and definitive host factors.<sup>16,40</sup>

This study shows significant associations between the location of the cows with fascioliasis and dicrocoeliasis. It has been reported that differences in parasite prevalence in cattle might be influenced by different geographical areas (ecology) and this is also associated with snail habitats in grazing areas of animals.<sup>14,16,19,41,42</sup> Fascioliasis has been particularly linked to climatic conditions of the area and a grazing history of animals.<sup>35</sup>

The study also recorded mixed trematodiasis. Similarly, mixed paramphistomiasis and fascioliasis have been reported elsewhere: in Ethiopia,<sup>19</sup> in Tanzania,<sup>17,30</sup> in Zambia,<sup>13</sup> in Nigeria,<sup>9</sup> and in Malaysia.<sup>34</sup> The co-infection of *Fasciola* with *Dicrocoelium* was also reported in Nigeria.<sup>39</sup> Co-infections might be due to different factors such as simultaneous exposure of cattle to different trematodes, probability of the different trematodes surviving in the same intermediate hosts, and similarities of some of the trematode life cycles.<sup>9</sup> Some metacercariae of different trematode species might be ingested together by cattle and cause co-infections. The mixed infections recorded in this study may suppress the host immune system predisposing it to other diseases and reduce livestock production. The co-infection could also lead to a high worm burden which would result in high egg contamination of the environment - this would increase the risk of contracting the zoonotic trematodes (*Fasciola* spp, *Echinostoma* spp) in humans. Indeed, both *Fasciola hepatica* and *F. gigantica* can cause zoonotic infections.<sup>43</sup> Although there is no data on human fascioliasis in Rwanda, human cases have been reported in a neighboring country (Tanzania).

The management and diagnosis of mixed trematodiasis are challenging: a host suffering from acute *F. hepatica* infection may not shed eggs in its faeces, and acute fascioliasis can be diagnosed with ELISA rather than fecal egg sedimentation. Major damages to the host suffering from acute fascioliasis is due to the migration of juvenile flukes from the intestine to the liver.<sup>44</sup> The fact that this study's cows were apparently healthy may indicate that the cows that were infected had chronic or asymptomatic trematodiasis: in ruminants, fascioliasis is predominantly chronic.<sup>45</sup> Again, adult *Paramphistomum* worms are non-pathogenic, but their migrating larvae may impact the host production and even cause death. Further, bovine dicrocoeliasis is also asymptomatic.<sup>44</sup>

Given that a sedimentation technique was used to differentiate trematodes based on their egg morphology; this might have resulted in misclassification of the trematodes. Although *Paramphistomum* spp eggs can be differentiated from those of *F. hepatica*,<sup>44</sup> eggs of *F. hepatica* can be mistaken for those of *Fasciolopsis buski* and *Echinostoma* spp.<sup>45</sup> According to Verocai et al,<sup>44</sup> eggs of *Dicrocoelium dendriticum* cannot be confused with those of other flukes because they are too small. However, given such eggs are brown, when they are detected using a sedimentation technique, the test is difficult to read due to excessive debris.<sup>44</sup>

Fascioliasis is refractory to praziquantel, while it is responsive to triclabendazole. Echinostomiasis can also be treated with praziquantel.<sup>46</sup> Nitroxinil is one of anthelmintics that are effective against *F. hepatica* and *Paramphistomum* spp.<sup>47</sup>



This study's major limitation was that the trematodes were identified at the genus level based on morphology of the eggs detected using a simple sedimentation technique. This diagnostic technique could miss some small eggs of *Dicrocoelium* spp. Molecular-based diagnostics would help to avoid misclassifying the trematodes.

## Conclusion

The overall prevalence of bovine trematodiasis in Nyagatare district, Rwanda was 69.0%. Different trematodes were identified including *Paramphistomum* spp, *Fasciola* spp, *Dicrocoelium* spp, and *Echinostoma* spp. The location of the cow was associated with the occurrence of bovine trematodiasis (mono or mixed fascioliasis and/or dicrocoeliasis). The high overall prevalence of trematodes in cattle in this study is adequate to limit and constrain cattle production leading to significant economic losses to small-scale farmers due to decreased productivity, treatment costs, and potential condemnation of affected organs during slaughter. Trematodiasis constitute a major hindrance to livestock production and human health : for example, *Fasciola* spp and *Echinostoma* spp are zoonotic. Therefore, different approaches should be applied to prevent and control the trematodiasis in cows and other livestock (sheep and goats) and reduce the risk of contracting fascioliasis and echinostomiasis in humans in Nyagatare district in Rwanda.

## Ethics Approval and Informed Consent

This study was concerned with live cows, not humans; therefore, study approval was given by the academic council of the School of Veterinary Medicine, University of Rwanda. Before collecting the data, the cattle farmers were briefed about the study, and only those who consented to admit their cows for faecal sampling were selected. Further, the cows were also treated with the best practice of veterinary care.

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## Disclosure

The authors report no conflicts of interest in this work.

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