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Original Research Article

Prevalence and Impact of Fasciola gigantica and Dicrocelium dendriticum Hepatic Co-Infections in Domestic Ruminants from Municipal Slaughterhouses of Diamare Division, Far North Cameroon

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Abstract: Domestic animals are very often subject to associated hepatic parasitic infestations, the consequences of which are little known on farms. The present study, which aimed to determine the prevalence and impact of hepatic co-infestation with *Fasciola gigantica* and *Dicrocelium dendriticum* in domestic ruminants in butcheries in the Diamaré department, was conducted from January to July 2023. The livers were necropsied by incising the ventral part of the parenchyma along the hepatic and bile ducts in order to find and collect the flukes with the naked eye. Of the 800 cattle, 175 sheep and 1008 goats inspected, only cattle showed co-infestation with an overall prevalence of 4.25% which is significantly lower than that due to singular *F. gigantica* infestation (15.62%) and higher than that due to *D. dendriticum* (1.37%) (χ 2=27.7; ddl=2; p < 0.000). These co-infections were higher in females (3.37%) (p<0.05), and in 6–7-year-old cattle (5.75%) (p>0.05). Their parasite densities were significantly lower than in cases of fasciolosis (24.75±18.21 flukes) but higher than in cases of dicroceliosis (8.11±6.89 flukes) (F=191.91; ddl=79; P < 0.000). The level of animal infestation was low for all types of infestation. The economic losses due to this *F. gigantica/D. dendriticum* co-infestation, evaluated at 7,650,000FCFA/year (12,584.25 us\$), are lower than those due to single *F. gigantica* infestations (28,116,000FCFA/46,250.82 us\$/year), and higher than those due to *D. dendriticum* (2,466,000FCFA//4,056.57 us\$/year). These results show that parasitic hepatic co-infestation with *F. gigantica/D. dendriticum* exists and remains a serious health and economic problem.

Keywords: Co-Infestation, Fasciola Gigantica, Dicrocoelium Dendriticum, Ruminants, Abattoirs, Economic Impact.

INTRODUCTION

Livestock production in Cameroon provides income for almost 30% of the rural population and contributes 20% to agricultural GDP (MINEPIA, 2011). Meat production is estimated at around 270,000 tons (MINEPIA, 2021), including 12,304 tons from cattle, 14,232 tons from goats and 7,260 tons from sheep in the Far North region (MINEPIA, 2021). However, this production is still insufficient to meet the animal protein needs of the population due to the lack of control over the breeding system, genetic and zootechnical limitations, and health problems associated with hepatic distomatosis, such as hepatobiliary fasciolosis and dicrocoeliosis (Ali *et al.*, 2011).

Hepatobiliary fascioliasis is a zoonotic parasitic disease caused by invasion of the liver and bile ducts by adult and larval forms of Fasciola trematodes (Braun et al., 1995). Clinically, it is manifested by edema, especially in the panniculus, pale and discolored mucous membranes, subicteric and oily eyes, and diarrhea (Razafindramanga, 2011; Razafindrabe, Dicrocoeliosis or small liver fluke is a parasitosis caused by infestation of the bile ducts by a trematode flatworm of the genus Dicrocoelium (Ait and Bellout, 2017). It affects ruminants and sometimes humans, causing diarrhea, emaciation, altered fleeces, altered general condition, and dilation of the central veins and periportal vessels (GDS, 2017; OSAV, 2021). These two parasitosis can cause chronic lesions such as cholangitis, which, if persistent, promote the development of fibrosis

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and cirrhosis of the liver, leading to sudden and significant morbidity and mortality (GDS, 2017; OSAV, 2021).

In Cameroon, and particularly in the Far North Region, the majority of studies on distomatosis have been conducted independently. A paucity of research has addressed the co-infections and consequences of the two parasitosis. The objective of the present study was to contribute to the understanding of the prevalence and impact of hepatic parasitic co-infections in ruminants in slaughterhouses of Diamaré municipality. Specifically, the objective was to

- Determine the prevalence of hepatic coinfestation;
- Determine the level of hepatic parasite on coinfestation;
- Assess the impact of hepatic parasitic coinfestation in ruminants.

1. MATERIALS AND METHODS

1.1 Study Area

The study took place from January to December 2023 in the main slaughterhouses in the Diamare division (Fig. 1).

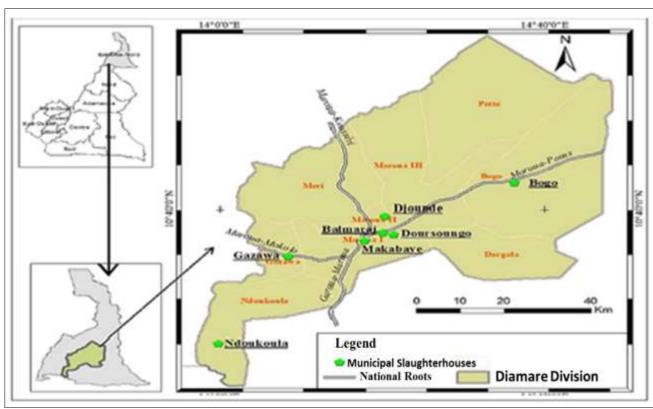


Figure 1: Map showing the location of the study area and sites

The Diamaré department, situated Cameroon's Far North region between 10° and 11° North latitude and 14° and 15° East longitude, encompasses an area of 4.666 square kilometers (INS 2019). Administratively, the department is divided into nine arrondissements, including Maroua I, Maroua II, Maroua III, Bogo, Gazawa, Meri, Petté, Ndoukoula, and Dargala. The prevailing climate is tropical, classified as Sudano-Sahelian, with a prolonged dry season extending from October to April and a concise rainy season from May to September. Rainfall is generally lower, averaging around 82.4 millimeters per year (Lucas et al., 2017). The hydrological network depends on the Lake Chad basin, with seasonal Mayo flows such as the Mayo-Tsanaga, Mayo-Boula, and Kaliao emptying into the Logone (INS 2018). The vegetation of the region consists of a grassy steppe, interspersed with a few thorny shrubs, such as Azardirachta indica, Acacia nilotica, A. oblida,

Mangifera indica, and Citrus lemon (Baye-Niwahl et al., 2020). The population is estimated at 864 (185 inhabitants/km2) and engages in agricultural activities such as the cultivation of cereals, including sorghum, maize, millet, and rice, as well as legumes, such as cowpeas, soybeans, groundnuts, and voandzou (INS 2019). Other crops cultivated include onions, potatoes, and sweet potatoes (INS, 2019). Livestock farming is predominantly focused on cattle, sheep, goats, and poultry, with pigs, horses, camels, donkeys, and beekeeping being practiced to a lesser extent (INS 2019).

1.2 Study Sites

The study was conducted in the municipal abattoirs of Maroua 1, Maroua 2, Maroua 3, Bogo, Gazawa, and Ndoukoula (Fig. 1). These abattoirs were selected due to their accessibility, the significance of

slaughtering large and small ruminants, and the availability of the personnel working there.

1.3. Sample Size

The study's sample size was determined using the Thrusfield formula: $N=Z2 \times P(1-P)/M2$, where N is the sample size, Z is the critical value for a confidence level of 95%, P is the estimated proportion of the population, M is the margin of error, and 5% was used for M.

 $N=Z^2 \times P (1-P)/M2$

Where: N=sample size; Z=95% confidence level, Z=1.96:

P=estimated proportion of the population (when unknown, P=0.5 is used);

M=tolerated margin of error (5% is used).

1.4. Liver Autopsy

This consists of carrying out macroscopic inspections of ruminant liver and bile to search for and collect parasites using the method described by Habiba (2015). The inspection begins with a macroscopic observation of both sides and the parenchyma, followed by an incision of the ventral part of the parenchyma along the hepatic and bile ducts to look for staves in the bile and hepatic ducts. The contents of the gall bladders of each animal are also sieved to collect any flukes present. Upon the observation of adult flukes, they are collected and placed in a plastic box. At the conclusion of the inspection, the parasites from each box are rinsed with water and placed in boxes containing 95% alcohol. In the laboratory, identification is based on morphological characteristics specific to each species. When two different parasites (F. gigantica and D. dendriticum) are identified in the same liver, the animal is considered to be co-infected. The parasite species identified were enumerated to ascertain the parasite load and the extent of infestation.

1.5. Determination of the Infestation Level

The infestation level is estimated based on the number of adult flukes and the parasite species. For F. gigantica adults, infestation is low if the number of flukes is \geq 20 parasites, medium if it is between 20-50 parasites, and high if it is more than 50 parasites (Boucheikhchoukh et al., 2012). For D. dendriticum adults, infestation is low if the number of flukes is <100 parasites, medium for 101-200 parasites, and high for

201-300 parasites (Camara *et al.*, 1996). For coinfestation, the infestation is combined with the two parasitic species. It is low if the number of flukes is between $(0-100) / (\le 20)$, average between (101-200) / (20-50), and high between $(201-300) / (\ge 50/)$.

1.6 Assessment of Economic Losses

The economic losses were calculated from infested or co-infested livers, and the estimated annual loss due to these parasites was calculated using the formula of Ogunrinade and Adegoke (1982): ALC=CSR x LC x P.

Where, ALC=annual loss; CSR=average annual number of ruminants slaughtered; LC=average cost of a liver; P=prevalence of infestation in slaughtered ruminants.

To do this, the average annual number of cattle, sheep, and goats slaughtered in these abattoirs was evaluated, and the selling price of the liver of each type of animal was known.

1.7. Statistical Analysis

The data collected during the field visit were meticulously compiled, transferred to Excel, and then subjected to rigorous analysis using Sphinx.V5 software. The collated data were meticulously calculated on the basis of percentages to determine prevalences and on the basis of averages to determine parasite loads. The χ^2 test was used to compare percentages and the ANOVA to compare means.

2. RESULT

2.1. Overall Prevalence

A total of 1,983 animals were examined in this study, including 800 cattle, 175 sheep, and 1,008 goats (Table 1). The results of liver and bile autopsies revealed infestation prevalences in cattle of 15.62% for *F. gigantica*, 1.37% for D. dendriticum, and 4.25% for *F. gigantica* - *D. dendriticum* co-infestation. These results indicate a statistically significant difference (χ^2 =27.7; ddl=2; p < 0.000), suggesting that cases of co-infestation are less prevalent than *F. gigantica* infestations, yet more prevalent than *D. dendriticum* infestations. In contrast, no *F. gigantica* or *D. dendriticum* infestations or co-infestations were observed in sheep and goats.

Table 1: Prevalence of co-infestation by species

Tuble 11 110 tubeled of co infection by species							
Méthode	Animaux	NE	F. gigantica	gigantica D. dendriticum Co-			
			NI(P)	NI(P)	NI(P)	χ^2	P value
Autopsie	Bovins	800	125(15.62)	11(1.37)	34(4.25)	27.7	< 0.000
	Ovins	175	00(00)	00(0,00)	00(00)		
	Caprins	1008	00(00)	00(0,00)	00(00)		
	Total	1983	125(15.62)	11(1.37)	34(1.71)		

NE=number of animals examined, NI=number of animals infested and P=prevalence in %.

2.2 Prevalence According to Intrinsic and Extrinsic Factors

2.2.1 Prevalence According to Slaughterhouses

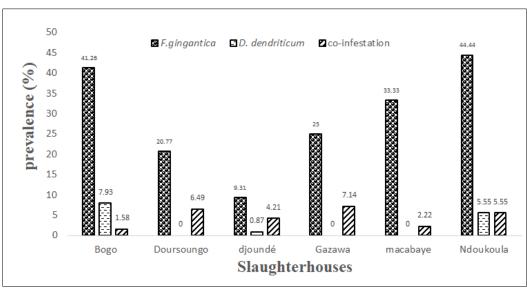


Figure 2: Prevalence of distomatosis co-infestation by slaughterhouses

As demonstrated in Figure 2, the prevalence of co-infections exhibited significant variations, ranging from 1.58% observed in the Bogo municipal slaughterhouse to 7.14% in the Gazawa abattoir $(\chi^2=17.7; ddl=2; p < 0.000)$. The Z-test indicates that cases of co-infestation were more prevalent in cattle from Gazawa (7.14%), followed by Doursoungou (6.49%) in Maroua, Ndoukoula (5.55%) and Djoundé in Maroua 1 (4.21%). A further analysis of infestation variations according to slaughterhouse reveals a highly significant relationship ($\chi^2=197.7$; ddl=2; p < 0.000) between coinfestation with F. gigantica/D. dendriticum (1.58%) and single-species infestation with F. gigantica (41.26%) at the Bogo abattoir. % for D. dendriticum and 20.77% for Doursoungo gigantica at the municipal slaughterhouse (χ^2 =231.6; ddl=2; p < 0.000), and

between 0.87% for *D. dendriticum* and 9.31% for *F. gigantica* (χ^2 =1669.91; ddl =2; p < 0.000), between 0.00% for *D. dendriticum* and 25% for *F. gigantica* (χ^2 =84.71; ddl=2; p < 0.000), and between 0.00% for *D. dendriticum* and 33.33% for *F. gigantica* (χ^2 =148.9 2; ddl=2; p < 0.000), and between 5.55% for *D. dendriticum* and co-infestation with *F. gigantica/D. dendriticum* and 44.44% for *F. gigantica* at the Ndoukoula municipal slaughterhouses (χ^2 =56.03; ddl=2; p < 0.000). At the Doursoungou, Djoundé, Gazawa, and Makabaye slaughterhouses, co-infection with *F. gigantica/D. dendriticum* was the second most prevalent hepatic parasitosis after hepatobiliary fasciolosis.

2.2.2 Prevalence According to Sex

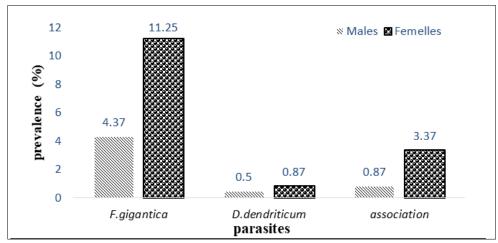


Figure 3: Prevalence of co-infestation according to sex

As illustrated in Figure 3, the prevalence of infestations exhibits variation according to sex, with

females demonstrating higher infestation rates compared to males in the context of *F. gigantica* infestation

(11.25%), *D. dendriticum* infestation (0.87%), and *F. gigantica/D. dendriticum* co-infestation (3.33%). However, a statistically significant difference was only observed in the cases of fasciolosis and co-infestation [$(\chi^2=4.26; ddl=2; P>0.05)$) and ($\chi^2=5.26; ddl=2; P>0.05$), respectively].

2.2.3 Prevalence by Age Group

The prevalence of cattle according to age (Table 2) reveals a notable prevalence of *F. gigantica* and *F.*

gigantica/D. dendriticum co-infections, with the highest incidence observed in adult cattle aged 6-7 years (1.87%), followed by those aged 4-5 years (1.12%) and 8-9 years (0.87%) as illustrated in Table 2. For dicrocoeliasis, infestation levels were observed to be highest in adult cattle aged 4-5 years (0.5%). However, these results did not demonstrate any statistically significant differences [(χ_r^2 =5.69; ddl=10; p > 0.05), (χ_d^2 =1.29; ddl=10; p > 0.05), (χ_c^2 =0.83; ddl=10; p > 0.05)].

Table 2: Prevalence of infestation in cattle as a function of age

Age	F. gigantica		D. dendriticum		Co-infestation	
	NI(P)	$\chi_f^2(\mathbf{P}\mathbf{v})$	NI(P)	$\chi_d^2(Pv)$	NI (P)	$\chi_c^2(\mathbf{P}\mathbf{v})$
< 4 ans	4 (0.5)	5.69	1(0.12)	1,29	0(00)	0.83
4-5 ans	35(4.37)	(>0.05)	4(0.5)	(>0.05)	9(1.12)	(> 0.05)
6-7 ans	46(5.75)		2(0.25)		15(1.87)	
8-9ans	20(2.5)		3(0.37)		7(0.87)	
10-11 ans	18(2.25		1(0.12)		3(0.37)	
> 12 ans	2(0.25)		0(00)		0(00)	
Total	125(15.62)		11(1.47)		34(4.25)	

NI=number of infested animals, P=prevalence in % and Pv=p-value

2.3 Parasite Densities and Level of Infestation in Animals

The findings in Table 3 illustrate variations in parasite densities and infestation levels in co-infected and co-infested beef cattle. In cattle co-infected with F. gigantica and D. dendriticum, the level of infestation was significantly low (55.88%/100%) (F= 191.91; ddl= 79; P< 0.000 Furthermore, parasite loads were determined to be 16.43 \pm 12.55 adult flukes, with F. gigantica

exhibiting a predominance of 24.75 ± 18.21 adult fluke compared to *D. dendriticum* at 8.11 ± 6.89 adult flukes. In cattle with single infestations of *F. gigantica* or *D. dendriticum*, the levels of infestation were found to be significantly low (59.2%; 100%, respectively) and the overall mean densities were 55.25 ± 6.75 adult flukes for *F. gigantica* and 13.91 ± 11.37 adult flukes for *D. dendriticum*.

Table 3: Parasite density and level of infestation in infested animals

Table 5. I draste density and level of intestation in intested animals								
	I	7. gigantica		Mean Density				
Level of infestation	NI (Taux in%)	Min	Max	Mean ± standard deviation				
≤20	74(59.2)	1	20	55.25±6,75c				
20-50	44(35.2%)	25	47					
≥50	7(5.6)	50	64					
D. dendriticum								
Level of infestation	NI (Taux)	Min	Max	Mean ± standard deviation				
0-100	11(100)	4	43	13.91±11,37b				
101-200	00(00)	00	00					
2001-300	00(00)	00	00					
Co-infestation F. gigantica/ D. dendriticum								
Level of infestation D(F)	NI _D (Taux)/ NI _F (Taux)	Mini F (D)	Max F (D)	Mean ± standard deviation				
≤20 (0-100)	19(55.88) /34(100)	4(1)	20(30)	16.43±12,55 (F/D)b				
20-50 (101-200)	13(38.23) /00(0,00)	23(00)	50(00)	24.75±18,21(F)				
≥50 (2001-300)	2(5.88) /00(0.00)	57(00)	64(00)	8,11±6,89 (D)				

Values that do not have the same letters are not significantly different at the 5% threshold (P<0.05).

Legend: NI=number of animals infested, Min=minimum, Max=maximum; and P-V=p-value, D= *D. dendriticum*, F= *F. gigantica*.

3.3 Economic Impact

The economic repercussions of *F. gigantica/D. dendriticum* hepatic coinfections, estimated to amount to 7,650,000 FCFA per annum (equivalent to 12,584.25 United States Dollars) in cattle slaughtered in the

Diamaré department, is greater than that of *D. dendriticum* dicroceliosis (2,466,000 FCFA or 4,056.57 \$us/year), but less than that of *F. gigantica* fasciolosis (28,116,000 FCFA or 28,116,000 \$us).

3. DISCUSSION

This study of hepatic parasitic infestations in domestic ruminants from slaughterhouses in the Diamaré Department demonstrates the occurrence of coinfestation with *F. gigantica* and *D. dendriticum* at an aggregate prevalence of 4.25%. This prevalence exceeds the figures reported by Ulayi *et al.*, (2007) in Nigeria (0.86%), yet falls short of the findings documented by Florence *et al.*, (2018) and Shinggu *et al.*, (2019), Ieren *et al.*, (2016) and Yaro *et al.*, (2022) in Nigeria (22.3%, 21.4%, 50.51% and 58.7%, respectively). Furthermore, Ali *et al.*, (2011), Petros *et al.*, (2013), and Magaji *et al.*, (2014) have documented infestations of Fasciola hepatica and *D. dendriticum* in cattle bile, often without overt signs or symptoms.

The prevalence of F. gigantica/D. dendriticum co-infestation (4.25%) is lower than that of F. gigantica alone (15.62%), but higher than that of D. dendriticum (1.47%). These results contrast with those reported by Shinggu et al., (2019), who observed co-infestation prevalences (21.4%) that exceeded those of single F. gigantica infestations (6.9%) and were lower than those of D. dendriticum (59%) in Nigeria, and those of Ulayi et al., (2007) in Nigeria, who report prevalences of coinfestation (0.86%) lower than those of F. gigantica (1.37%) and D. dendriticum (35.4%) in Nigeria. Ait and Bellout (2017) reported low prevalences of F. gigantica fasciolosis in Algeria (0.70%), while high prevalences were highlighted by Bacha (2020) in Algeria (7.04%), Mwabonimana et al., (2009) in Algeria (6.7%), and Mohamed et al., (2021) in Tanzania (12.3%), Petros et al., (2013) in Ethiopia (21.9%), Menya (2017) in Adamaoua, Cameroon (63%), Massamba (2020) in Burkina (67%), and Merdas (2015) in Algeria (21.92%). In contrast, lower prevalences of D. dendriticum dicrocoeliosis were observed by Mohamadzadeh et al., (2015) in Iran (0.6%) and Mohamed et al., (2019) in Algeria (0.52%), while higher Conversely, higher prevalences were reported by Baudin (2005) in Haute-Saône (7.2%), Shinggu et al., (2019) in Nigeria (80.5%), and Mamatkulova (2021) in Uzbekistan (62.4%). The high prevalence of F. gigantica can be linked to the presence of ecological biotypes that are favorable to its intermediate host, Lymnaea natalensis.

When examining variations in infestations according to sex, co-infestation with *F. gigantica* and *D. dendriticum* was observed to be higher in females (3.37%) than in males (0.87%). Florence *et al.*, (2018) also reports higher co-infestation with *F. gigantica* and *D. dendriticum* in females (24.5%) than in males (20.0%) in Nigeria. The higher prevalence of *F. gigantica* infestation in females (11.25%) has also been documented by Bacha (2020) in Algeria and Siama and Njan-Nlôga (2018) in the Far North region of Cameroon. According to Howell *et al.*, (2015), the high prevalence observed in females can be attributed to the fact that, in herds, females are more numerous and have greater physiological needs (heat, conception, milk production,

calf nutrition) which force them to feed more than males and to be more exposed to parasitosis.

The present study found that cattle aged between 6 and 7 years old exhibited the highest risk of infection with F. gigantica/D. dendriticum (1.87%) and F. gigantica (5.75%). In contrast, the prevalence of a single infestation with D. dendriticum was highest in cattle aged 8 to 9 years (0.37%). This finding aligns with the results reported by Linda (2019) in northern Algeria, who observed that animals over 4 years of age are the most affected by dicrocoeliosis (0.25%). Murat et al., (2009) reported that older cattle are more susceptible (12.7%) to dicrocoeliosis than younger cattle (3.04%) in Turkey. Higher prevalences in older animals were also reported by Bacha (2020) in Algeria (60%), Mebark and Megane (2018) in Algeria (57.14%), and Petros (2013) in Algeria (31.78%), while young animals were more infested (18.05%) than adults. According to Yaro et al., (2022), the higher prevalence in older cattle could be attributed to prolonged exposure to infection, resulting in the weakening of their immune systems over time.

The animals demonstrated minimal infestation levels in cattle with co-infestation (59.2%), fasciolosis (55.88%), and dicrocoeliosis (100%). These results are consistent with those reported by Siama and Njan Nlôga (2018) in Cameroon, Mebark and Megane (2018) in Algeria, Shinggu *et al.*, (2019) in Nigeria, Ieren *et al.*, (2016) and Yaro *et al.*, (2022) in Nigeria. According to Petros (2013), this low level of infestation can be attributed to the acquired immunity of the animals, which limits the number of flukes and dunce during reinfestations through humoral immune responses and hepatic tissue reactions.

The mean parasite density (16.43 ± 12.55) flukes) in animals with coupled F. gigantica/D. dendriticum infestations was lower than that of single fasciolosis cases (55.25 ± 6.75 flukes) and comparable to that of animals infested only with D. dendriticum (13.91 ± 11.37 flukes). A subsequent examination of the stave composition of co-infested animals revealed a predominance of F. gigantica adults (24.75 \pm 18.21 staves) over those of D. dendriticum (8.11 \pm 6.89 staves). suggesting the presence of interspecific competition. These results are consistent with those reported by Mohamed et al., (2021), who observed low levels of infestation by F. hepatica and Paramphistomum sp. Equar and Gashaw (2012), also proposed that interspecific competition, which can manifest as a limitation on the number and size of the weakest parasites, may underlie these observations.

The annual economic losses incurred as a result of liver condemnation in cattle afflicted with coinfestations amount to 7,650,000 F CFA (equivalent to 12,584.25 USD). This figure stands in contrast to the losses associated with a singular *F. gigantica* infestation,

which are estimated at 28, 116,000 F CFA/46,250.82 \$ us) but higher than for D. dendriticum infestations (2,466,000 F CFA/4,056.57 \$ us). These results are consistent with those reported by Ali et al., (2011) for ruminants infested with the combination of fasciolosis (142,763,223.87 and dicrocoeliosis \$234,845.50). These financial losses are notably lower than those reported by Petros et al., (2013) in Ethiopia due to fasciolosis (740,792,466 FCFA/1,218,603.61 \$us) and Razanajatovoarivelo (2015) in Madagascar due to seizure (1443540 FCFA/2,374.62 \$us), and Mohamed (2021) in Madagascar (1298281495 FCFA/2,135,673.06 \$us). Ethiopia (708929.28 FCFA/1,166.19 \$us), and Mohsen et al., (2018) for dicrocoeliosis in Iran (5,687,301.672 FCFA/9,355.61 \$us). The elevated costs observed can be attributed to the significant number of animals that were slaughtered. These animals originated from flood plains, where ecological conditions are conducive to the survival and proliferation of intermediate host mollusks the subsequent and transmission of the disease to animals.

4. CONCLUSION

The present study demonstrated that the hepatic parasites F. gigantica and D. dendriticum, which are responsible for fasciolosis and dicrocoeliosis in cattle. may coexist, with a prevalence of 4.25%, compared with 15.62% for F. gigantica and 1.37% for D. dendriticum. The overall level of infestation was low in all cases. The parasitic load in co-infested animals was found to be lower than that caused by F. gigantica, but higher than that caused by D. dendriticum. The annual economic losses due to co-infestation were found to be lower than those associated with liver seizures caused by parasitosis unique to F. gigantica, but higher than those associated with D. dendriticum. These findings indicate the existence of interspecific hepatic relationships between F. gigantica and D. dendriticum, characterized by competition for space, resulting in a reduction in their numbers. Consequently, further research in the form of serological studies is necessary to elucidate the immunological elements and mechanisms underlying this competitive relationship between the two parasites.

Statements and Declarations

Funding: No funding was received for conducting this study.

Competing Interests: This study was conducted without any conflict of interest.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Augustin SIAMA, IBRAHIM, and Alexandre Michel NJAN NLÔGA. The first draft of the manuscript was written by Augustin SIAMA and IBRAHIM and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Approval: No applicable.

Consent to Participate: No applicable.

Consent to Publish: No applicable.

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