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Original Research Article

Effectiveness of Screening for Hepatobiliary Fascioliasis Using the Mini-Flotac Kit among Slaughterhouses Cattle from the City of Ngaoundere, Adamawa, Cameroon

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Abstract: Hepatic fasciolosis is a gastrointestinal helminthiasis caused by a parasite of the genus Fasciola that severely impacts the health of animals and humans, and for which direct diagnosis remains ineffective. The study was conducted from January to July 2023 on 400 cattle from municipal slaughterhouses in the city of Ngaoundere to determine the effectiveness of screening for bovine fascioliasis using the Mini-FLOTAC test. The results showed a prevalence of 33.58% in the Mini-FLOTAC test, which is comparable to that of coprology by sedimentation (30.75%) (p<0.05) but significantly lower than that obtained during bile examination (67%) and liver autopsy (46.50%) (p< 0.001). The parasite densities, which vary significantly between 10.72±1.6 flukes for liver autopsy and 688.6±10.7 eggs for bile examination (p< 0.01), show no significant difference between that obtained in coprology by sedimentation (254) and in the Mini-FLOTAC test (11.82) (p>0.05).Infestation levels varied between 1.8% and 79.8% for sedimentation coprology, 9.5% and 90.5% for Mini-FLOTAC flotation coprology, 3.2% and 78% for liver autopsy, and 5.8% and 50.8% for bile examination are significantly low (p>0.05). The liver autopsy test is highest significant sensitive (69.40%), followed by the flotation/Mini-FLOTAC coprological test (50.17%), and the sedimentation coprological (45.90%) (p< 0.001). The Mini-FLOTAC test is therefore more sensitive than coprology by sedimentation for the determination of prevalence of fascioliasis.
Keywords: Fasciolosis, Cattle, Screening, Coprology, Sedimentation, Mini-FLOTAC Test.

INTRODUCTION

Hepatobiliary fasciolosis is a parasitic disease caused by the migration and localization of larval and adult forms of the genus *Fasciola* in the liver (Andriamanantena *et al.*, 2005). It affects domestic and wild animals, causing enormous economic losses estimated at over 200 million CFA francs per year in Cameroon (Ashrafi *et al.*, 2014). Other indirect losses such as metabolic deviation, growth retardation, decreased fertility, decreased immunity, and increased susceptibility to bacterial infections have also been observed (Ayadi *et al.*, 1991; Ashrafi *et al.*, 2014). Fasciolosis is also a foodborne zoonosis, as it can be transmitted to humans through the consumption of aquatic or moist plants infested with metacercariae (Mas-Coma *et al.*, 1999).

Despite significant progress in diagnosis and treatment, the socio-economic impact and prevalence of disease remain very high in several African countries (Mekroud, 2002; Mwabonimana, 2009; Abakar, 2014; Mebarka *et al.*, 2018). In Cameroon, serological diagnostics are very expensive and inaccessible. However, despite their low effectiveness, coprological tests remain the only means of providing information on the health of grazing animals. Numerous studies, such as that by Simo *et al.* (2020), have evaluated the effectiveness of sedimentation coprology for screening for fasciolosis. To this end, a Mini-FLOTAC kit has been developed for the rapid and effective screening of helminth eggs in animals and humans. The question is whether this kit could improve the effectiveness of coprological diagnosis in screening for hepatobiliary fasciolosis. Our study aims to evaluate the effectiveness of this new coprological method using the Mini-

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FLOTAC kit in screening for fasciolosis in beef cattle in the city of Ngaoundere. More specifically, it will involve:

- Assess the prevalence of fasciolosis using coprology with the Mini-FLOTACT Kit;
- Assess the level of infestation using coprology with the Mini-FLOTACT Kit;
- Determine the effectiveness of coprology using the Mini-FLOTAC Kit.

1. MATERIALS AND METHODS

1.1. Study Area

This study was conducted was conducted from January to July 2023 in the city of Ngaoundere, capital of the Adamawa Region (Figure 1).

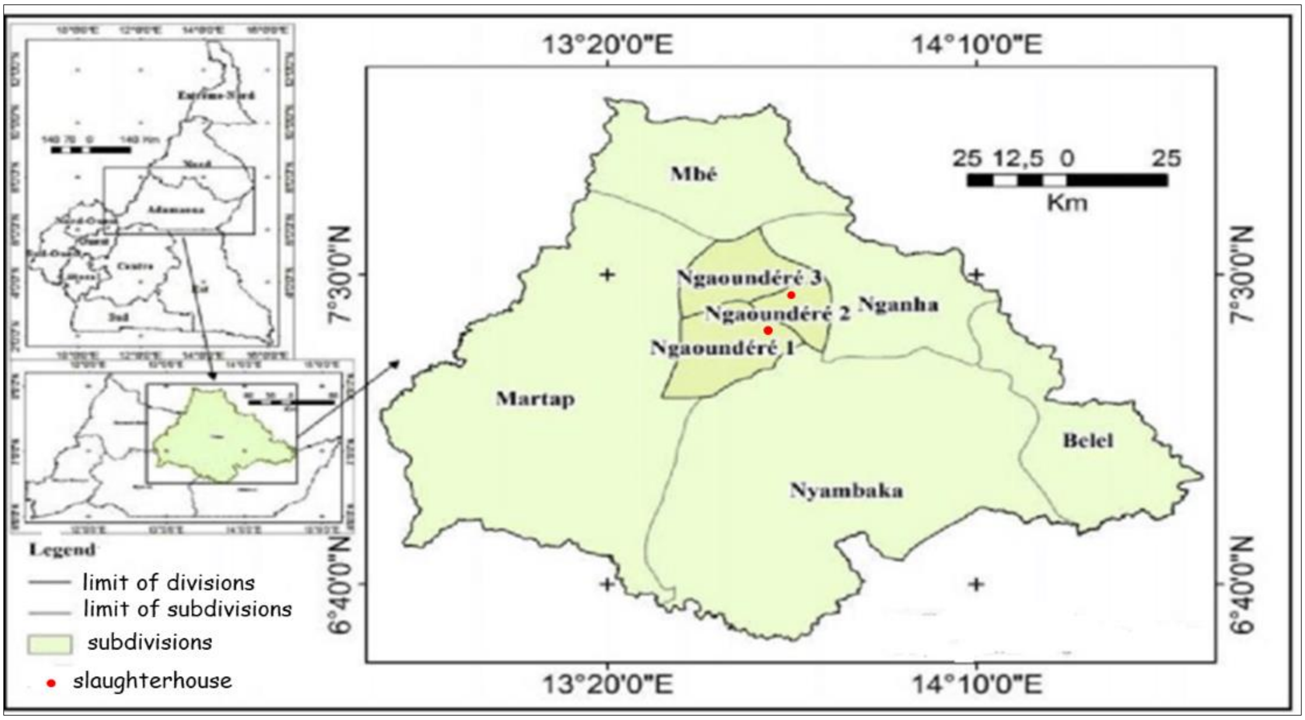

Figure 1: Location map of municipal slaughterhouses in the city of Ngaoundere

The city of Ngaoundere is located between 7o 3' and 7o 32' north latitude and 13o 20' and 13o 54' east longitude (Tchotsoua *et al.,* 1999). It covers an area of approximately 2,500 km² and is dominated by granite massifs with several peaks, such as Mount Ngaoundere and Mount Ngaoun-Nday, and volcanic accumulations (Tchotsoua *et al*., 1998). The climate is tropical Sudanese, with a rainy season lasting seven months (March to October) and a short dry season from November to March. Average annual rainfall is around 1,500 mm (Tchotsoua *et al*., 2002). Average annual temperatures vary between 22 and 25°C (Tchotsoua *et al.,* 1999). The hydrographic system consists of watercourses such as the Vina (Tchotsoua *et al.,* 2002).

1.2. Sampling Sites

Samples were taken from cattle at the municipal slaughterhouses of Ngaoundere II in Baladji 2 and Ngaoundere III in Dang. These slaughterhouses were chosen because of their accessibility, the importance of ruminant slaughter and the availability of the people working there.

1.3. Sampling

The cattle were selected at random based on their arrival at the slaughterhouse. Using a black ink marker, a mark was placed on one of the horns of the selected animal to enable traceability before and after slaughter. In addition, identification tags with pins and numbered from 1 to 10 or more were designed for the identification and traceability of the livers of the animals selected for post-mortem examination. The maximum sample size was calculated with a 95% confidence interval and 5% precision using the method described by Thrusfield (2007).

$$n = \frac{z^2 P(1-P)}{d^2}$$

Where n = sample size; P = apparent prevalence = 50%; d = desired absolute precision = 5%. Applying the formula below with an apparent prevalence of 50% gives a minimum sample size of 384 cattle.

1.5 Sampling Analysis

In this study, we will compare coprology using the Mini-FLOTAC Kit with coprology using sedimentation, using the results of liver autopsies and microscopic examinations of bile as positive controls.

1.5.4 1.5.4 Floatation Coprology using the Mini-FLOTAC Kit

Floatation coprology using the Mini-FLOTAC kit is a type of coprology that requires a zinc sulphate solution. To prepare the flotation or zinc sulphate solution, 685 g of zinc sulphate was added to 685 ml of water and dissolved using a spatula or magnetic stirrer. To perform this test, 45 ml of flotation solution was placed in the Fill-FLOTAC container. Then, 5 g of faecal matter, previously mixed thoroughly with the spatula, was placed in the Fill-FLOTAC conical collector until it was full. The Fill FLOTAC was then closed and the stool suspension was homogenized by pumping the conical collector up and down 10 times in the container, while turning it to the right and left. A tip was placed on the side hole of the Fill-FLOTAC and the Fill-FLOTAC was inverted 5 times to mix the sample in order to fill the flotation chambers of the Mini-FLOTAC. After 10 minutes of rest, the reading disc is turned 90° clockwise with the key until the reading disc stops, to separate the floating parasitic elements from the stool debris. The key is then removed and the Mini-FLOTAC examined under a microscope. The multiplication factor used to obtain the number of eggs per gram of stool is 10 for one chamber. The number of eggs per gram (OPG) was calculated using the following formula: OPG = n x 10, where n = number of eggs in one chamber of the Mini-FLOTA slide and 10 = multiplication factor.

1.5.1. Autopsy of the Livers

Liver autopsies were performed according to the methods described by WHO (2021) to search for adult flukes. They begin with visual observations of the liver surfaces, followed by an incision in the ventral part of the parenchyma along the hepatic and biliary ducts and the collection of adult flukes. A bovine animal is considered infested when residues or at least one fluke are observed (CATHA, 2005). The flukes present in infested livers are removed and counted. Prevalence is the ratio of the number of infested animals to the sample size.

1.5.2. Gallbladder Examination

In the laboratory, the bile was placed in a stemmed glass. After 15 minutes of sedimentation, the sediment was aspirated with a pipette and then deposited in a Petri dish to a height not exceeding 1 mm to facilitate observation of the fluke eggs **(**Kaufmann, 1996). The solution was stained with a few drops of methylene blue. *F. gigantica* eggs were searched for under an electric microscope. To determine the number of eggs per ml (OPL), the following formula was used: OPL = n x 100; where n = number of eggs in one chamber of the McMaster slide **(**Kaufmann, 1996).

1.5.3. Sedimentation Coprology

Simple sedimentation coprology was used to estimate the prevalence of infestation and parasite density **(**Kaufmann, 1996). To do this, 5 g of faeces were weighed using electronic scales and mixed with 70 ml of distilled water. The mixture was placed in a mortar, homogenized with a pestle, then filtered using a tea strainer and sedimented for 1 hour in a 250 ml conical glass (height = 15 cm). A pipette was used to gently remove the sediment. The suspension was stained with a few drops of methylene blue and observed under an electric microscope. A few drops of the remaining mixture were collected using a pipette and placed on a McMaster slide for egg counting under the microscope. To calculate the number of eggs per gram of faeces (OPG), the following formula was applied: OPG = n x 100 Where n = number of eggs counted in one chamber of the McMaster slide **(**Kaufmann, 1996).

2.5 Assessing the Level of Infestation

To determine the level of infestation in the autopsied livers, the flukes from each liver were collected in plastic jars and counted. Depending on the number of flukes, infested cattle were classified by type of infestation level according to the classifications described by (WHO, 2021). Infestation is low if the liver contains no more than 20 flukes, medium if there are 20 to 50 flukes, and high if there are more than 50 flukes. In the case of sedimentation coprology and bile examination, the level of infestation is low when fewer than 400 eggs/g of faeces and 400 eggs/ml of bile are counted respectively, moderate when between 400 and 1,000 eggs/g of faeces or 400 and 1,000 eggs/ml of bile are counted, and severe when there are 1,000 to 2,500 eggs/g of faeces or 1,000 to 2,500 eggs/ml of bile. g of faeces or 400 and 1000 eggs/ml of bile, high for 1000 to 2500 eggs/g of faeces or 1000 to 2500 eggs/ml of bile, and massive for more than 2500 eggs/g of faeces or more than 2500 eggs/ml of bile ml. For the Mini-Flotac technique, the infestation level is low if the number of eggs is less than 50 eggs/g of faeces, moderate if it is between 50 and 500 eggs/g of faeces, high for 500 to 2500 eggs/g of faeces, and massive if the number of eggs is greater than 2500 eggs/g of faeces (WHO, 2021).

2.6 Assessing the Effectiveness of the Test

Sensitivity was assessed by comparison with bile examination, considered the gold standard technique (WHO, 2021).

$$\text{Sensibility} = \frac{\mathbf{VP}}{\mathbf{(VP+FN)}} \text{x}100$$

Where VP = number of positive cases in the test, FN = Positive number of reference test - VP. For our study, the reference test is the examination of eggs in bile.

2.7 Statistical Analyses

The data collected during the fieldwork was compiled using data collection sheets, entered into Excel 2016, and analysed using SPSS version 16.0 software. All data collected was calculated on the basis of percentages and averages. It was interpreted using Khi 2, Schwartz (Z), ANOVA and Duncan tests. The Khi 2 test allows percentages to be compared, while the Z test allows the significance of prevalence's to be compared in pairs. ANOVA allows averages to be compared and the Duncan test allows these averages to be ranked according to their significance.

2. RESULTS

2.1.1. Overall Prevalence of Fasciolosis

Throughout the study, 875 cattle were investigated (Table 1). The results show that 33.58% of cattle tested positive using the Mini-FLOTAC coprology kit, compared with 30.75% for sedimentation coprology, 46.5% for liver autopsy, and 67% for bile examination. Overall, these results show a very significant difference (χ2= 172.3; ddl= 3; p= 0.001). The Z test shows that bile examination, followed by liver autopsies, reveal the highest infestation rates, while Mini-FLOTAC and sedimentation coprology tests reveal lower rates that are not significantly different.

Table 1: Prevalence of fasciolosis according to different tests

Examinations	NE	NP	P (%)	CI	P value
Autopsy of the livers	875	407	46,5b	[41,59-51,41]	0,001
Sedimentation coprology	875	269	30,75a	[26,21-35,29]	
Coprology by Mini-FLOTAC kit	875	294	33,58a	[20,97-29,53]	
Gallbladder examination	875	586	67c	[62,37-71,63]	

Values followed by the same letters do not show significant differences at the 5% threshold.
Legend: CI: confidence interval at 95%; NE: Number Examined; NI: Number Positive; P: prevalence

3.2. Prevalence According to Intrinsic and Extrinsic Factors

3.1.1.2.6. Prevalence of Cattle Infestation According to Sex

The prevalence of infestation in animals obtained during bile examinations, liver autopsies, sedimentation coprology, and Mini-Flotac testing (Table 2) was higher in females (69.6%, 43.5%, 31.8%, and 35.01%, respectively) than in males (66.2%, 47.4%, 27.2%, and 28.86%, respectively). However, these differences are not statistically significant across the different tests (χ2= 0.36, ddl= 2, p= 0.55; χ2= 0.43, ddl= 2, p= 0.51; χ2= 0.72; ddl= 2; p= 0.38; χ2= 0.78; dl= 3; p= 0.38 respectively).

3.1.1.2.3. Prevalence by Breed

The findings on infestation by breed (Table 2) vary between 63.9% in Bokolo cattle breed to 68.3% in crossbred cattle for bile examination, between 41.7% in Bokolo cattle breed and 48.6% in M'bororo cattle for liver autopsy, between 31.99% in Goudali cattle breed and 39.86% in crossbred cattle for Mini-FLOTAC coprology, and between 29.6% in M'Borolo cattle breed and 33.3% in crossbred cattle for sedimentation coprology. However, these variations are not significant (P > 0.05).

3.1.1.2.4. Prevalence by Weight

The data in Table 2 show that the prevalence of infestations in cattle varied according to weight, with no significant differences between 27.06% in animals weighing >200 kg and 30% in those weighing <150 kg for coprology by Mini-FLOTAC (χ2=0.825; ddl= 2; p= 0.527), between 59% in animals weighing >200 kg and 68% in those weighing <150 kg for bile examination (χ2=0.209; ddl= 2; p= 0.976), between 43.6% in those weighing >200 kg and 46.8% in those weighing less (<150 kg) for liver autopsy (χ2=3.621; ddl= 2; p= 0.016), between 28.2% in those weighing >200 kg and 33.3% in those weighing <150 kg for sedimentation coprology (χ2=0.213; ddl= 2; p= 0.662).

Table 2: Distribution of prevalence's according to intrinsic and extrinsic factors

Test	Autopsy of the livers			Sedimentation test			Mini-FLOTAC test			Gallbladder test		
	NE	NI	P (%)	NE	NI	P (%)	NE	NI	P (%)	NE	NI	P (%)
Sex												
Female	201	87	43.5	201	55	27.2	201	58	28.86	201	140	69.6
Male	674	319	47.4	674	214	31.8	674	236	35.01	674	446	66.2
Khi-deux (P-value)	0.43 (0.51)			0.72 (0.39)			0.78 (P=0.38)			0.36 (0.55)		
Races												
Goudali	347	159	45.9	347	107	30.8	347	111	31.99	347	234	67.3
M'bororo	311	151	48.6	311	92	29.6	311	102	32.80	311	208	66.9
Bokolo	79	33	41.7	79	24	30.6	79	26	32.91	79	50	63.9
Métis	138	63	46	138	46	33.3	138	55	39.86	138	94	68.3
Khi-deux (P-value)	0.615(0.893)			0.290 (0.962)			0.986 (0.805)			0.209 (0.976)		
Weight (Kg)												
<150	66	31	46.7a	66	22	33.3a	66	26	39.39a	66	44	66.7a
[150-200]	724	339	46.8a	724	223	30.8a	724	245	33.84a	724	492	68a
>200	85	37	43.6a	85	24	28.2a	85	23	27.06a	85	50	59a
Khi-deux (P-value)	0.147 (0.929)			0,213 (0,899)			0.825 (0.662)			1.128 (0.527)		

Test	Autopsy of the livers			Sedimentation test			Mini-FLOTAC test			Gallbladder test		
	NE	NI	P (%)	NE	NI	P (%)	NE	NI	P (%)	NE	NI	P (%)
Age (Year)												
<2	35	9	25b	35	4	12.5b	35	3	8.57b	35	20	56.2b
[2-4]	79	33	41.7b	79	22	27.8a	79	26	32.91a	79	57	72.2a
>4	761	365	48a	761	243	31.9a	761	265	34.82a	761	510	67a
Khi-deux (P-value)	3.621 (0.016)			2.867 (0.023)			3.211 (0.020)			1.281 (0,04)		
BCN												
] 0-1]	123	39	31.71c	123	43	34.96a	123	32	26.02a	123	57	46.34c
]1-3[	273	116	42.49b	273	80	29.30a	273	92	33.70a	273	158	57.88b
[3-5]	479	252	52.61a	479	146	30.48a	479	174	36.33a	479	371	77.45a
Khi-deux (P-value)	7.575 (0.019)			0.677 (1.38)			2.391 (0.393)			11.90 (0.006)		
Season												
Dry	329	134	40.7	329	105	32	329	116	35.26	329	221	67.3
Rainy	546	273	50	546	164	30	546	178	32.60	546	365	66.8
Khi-deux (P-value)	2.63 (0.04)			0.176 (0.675)			0.255 (0.613)			0.0019 (0.913)		

Legend: NE: Number Examined; NI: Number Positive; P: prevalence

3.1.1.2.5. Prevalence by Age

With the distribution of infestation according to age (Table 2), the prevalence of fasciolosis varied significantly between 56.2% in calves (<2 years) and 72.2% for bile examination (χ2=1.281; ddl=2; p=0.04), and between 25% in calves (<2 years) and 48% in adults (>4 years) for liver autopsy (χ2= 3.621; ddl= 2; p= 0.016), between 8.57% in calves (<2 years) and 34.82% in adults (>4 years) for coprology by Mini-FLOTAC with (χ2= 3.211; ddl= 2; p= 0.020), and between 12.5% in calves (<2 years) and 31.9% for sedimentation coprology with (χ2=2.867; ddl= 2; p= 0.023).

3.1.1.2.7. Prevalence Based on Body Condition Note (BCN)

The infestation rates in cattle according to Body Condition Note (BCN) (Table 2) varied significantly between 46.34% in cattle with BCN =] 0-1] and 77.45% in those with BCN = [3-5] for bile examination (χ2=11.9; ddl= 2; p< 0.01) and significantly between 31.71% in cattle with BCN =] 0-1] and 52.61% in those with BCN = [3-5] for liver autopsy (χ2=5.532; ddl= 2; p< 0.05). However, these rates vary without showing significant differences between 29.3% in cattle with BCN =] 1-3] and 34.96% in those with BCN =] 0-1] for coprology by sedimentation (χ2=0.677; ddl= 2; p= 0.048), and between 26.02% of cattle with BCN =] 0-1] and 36.33% of those with BCN = [3-5] for coprology by Mini-Flotac (χ2=2.391; ddl= 2; p> 0.05).

3.2.1 Prevalence by Season

The findings in Table 2 show that the prevalence of infestation in cattle varies without significant differences between 50% during the rainy season and 40.7% during the dry season for liver autopsies (χ2= 2.63; ddl= 1; P >0.05), between 30% in the rainy season and 32% in the dry season for sedimentation coprology (χ2= 0.176; ddl= 1; P >0.05), between 35.26% in the dry season and 32.6% in the rainy season for coprology by Mini-FLOTAC (χ2= 0.255; ddl= 1; P >0.05), and between 66.8% in the dry season and 67.3% in the rainy season for bile examination (χ2= 0.0019; ddl= 1; P >0.05).

3.1.2. Evaluation of Parasitic Densities

3.1.2.1. Overall Parasitic Densities

The data in Table 3 show the average densities of *F. gigantica* eggs according to the different types of tests. This density varies significantly between 11.82±1.6 eggs/g of feces for coprology using the Mini-Flotact test and 688.6±10.7 eggs/g of feces for bile examination (F= 101.751; ddl= 3; p= 0.002). Duncan's test shows that more parasite eggs were counted in coprology by sedimentation and bile examination than by Mini-FLOTAC.

Table 3: Overall densities of Fasciolosis by type of examination

Examinations	NE	Mean ± sd	CI	P-value
Autopsy of the livers	875	10,72±21,6^{a}	[9-22.36]	0.002
Sedimentation coprology	875	25±36,6^{b}	[19-38.9]	
Coprology by Mini-FLOTAC kit	875	11,82±22,9^{a}	[8.9-24.7]	
Gallbladder examination	875	688,6±100,7^{c}	[62.37-71.63]	

Legend: CI: confidence interval at 95%; NE: Number Animal Examined; sd= standard deviation

3.1.2.2. Assessment of Parasitic Loads According to Factors

3.1.2.2.1. Assessment of Parasitic Loads by Districts

The data in Table 4 show the egg densities assessed using different methods in the districts. It shows that these densities the average density of flukes ranges from 10.50 to 10.86 eggs/gf for liver autopsies, with no significant difference. However, the average densities of *F. gigantica* eggs range from 204.6 to 283.6 eggs/gf in sedimentation coprology, from 11.33 to 12.12 eggs/gf in coprology by Mini-FLOTAC, and from 662.2 to 732.6 eggs/gf in bile examination, but without any significant difference in this distribution (P > 0.05).

Table 4: Distribution of parasitic density depending on intrinsic and extrinsic factors parasitic loads

Test	Autopsy of the livers		Sedimentation		Mini-FLOTAC test		Gallbladder examination	
	Moats	CI	EPG	IC	EPG	CI	Egg per mL	CI
Sub-division								
Ngaoundere II	10.86±1.7	[8.6-13]	23.6±4.2	[19.1-37.6]	12.12±2.9	[8.4-15.8]	662.2±103.7	[532-791]
Ngaoundere III	10.50±1.5	[8-12.9]	28.6±9.4	[12.4-28.4]	11.33±2.8	[6.5-16]	732.6±114.8	[547-918]
	F=0.44 (P=0.833)		F=1.338 (P=0.248)		F=0.066 (P=0.797)		F=0.399 (P=0.528)	
Season								
Dry	11,88±1.8	[8.9-14.8]	28.3±7.1	[174-404]	11.8±2.5	[7.6-16]	812.6±121.1[b]	[617-1008]
Rainy	10.03±1.5	[8-11.9]	23.8±6.9	[154-311]	11.84±3.1	[7.8-15.8]	614.2±98.7[a]	[491-737]
	F=1.157 (P=0.28)		F=0.685 (P=0.41)		F=0.001 (P=0.99)		F=3.184 (P=0.047)	
Breed								
Goudali	11.07±1.6	[8.5-13.6]	23.3±6.5	[140-336]	10.12±2.5	[6.1-14]	769.8±118	[584-955]
M'bororo	12.42±1.9	[9-15.7]	26.4±5.6	[137-388]	13.23±3.3	[7.7-18.7]	687.6±116	[494-880]
Bokolo	7.25±1.1	[3.3-11]	27.7±8.8	[44-510]	14.44±3.2	[3.5-25.4]	569.4±75.3	[314-824]
Mixed Breed	8.01±1.1	[5-10.9]	25.7±4.4	[134-383]	11.42±2.9	[4-18.8]	553.9±69.4	[379-728]
	F=1.593 (P=0.191)		F=0.055 (P=0.983)		F=0.379 (P=0.768)		F=0.77 (P=0.511)	
Weight (Kg)								
<150	13.63±1.9	[6.4-20.8]	45±9.5	[104-795]	21±3.6	[7.2-34.7]	109.3±15.2	[52-1663]
[150-200]	10.33±1.6	[8.6-12]	23.4±6.4	[167-307]	10.96±2.8	[7.8-14]	659.4±103	[547-770]
>200	11.79±1.9	[5.4-18.1]	21.6±3.8	[69-418]	12.05±3.1	[1.8-22.2]	625.6±105	[284-966]
	F=0.623 (P=0.537)		F=1.430 (P=0.240)		F=1.588 (P=0.206)		F=2.309 (P=0.101)	
Age (Year)								
<2	6.43±1.3	[0.5-13]	37.5±1[a]	[20.4-95]	1.25±0.5[a]	[1-3.9]	550±11[a]	[39-1139]
[2-4]	9.47±1.4	[4.6-14.2]	20±4.9[b]	[61-338]	11.66±2.6[b]	[2.2-20.4]	641.6±82[b]	[363-920]
>4	11.05±1.7	[9.2-12.8]	26.5±6.9[b]	[196-342]	12.32±3	[9-15.5]	699.8±110[b]	[583-816]
	F=0.696 (P=0.499)		F=1.075 (P=0.042)		F=1.073 (P=0.043)		F=0.184 (P=0.032)	
Sex								
Male	8.04±1.3[a]	[5-10.7]	19.5±4.9	[84-25.4]	7.71±1.9	[3.5-12]	604,8±86	[426-783]
Female	11.52±1.7[b]	[9.5-13]	23.2±71.7	[19.8-35.9]	13.05±3.1	[9.4-16.6]	713,6±113	[586-841]
	F=3.104 (P=0.049)		F=1.954 (P=0.163)		F=2.311 (P=0.129)		F=0,718 (P=0,397)	
BCN								
] 0-1]	12.7±1.8[b]	[9.4-16]	35.1±8.5[b]	[211-529]	14.9±2.9	[9-20.8]	912.8±138[b]	[666-1158]
]1-3[	9.8±1.5[a]	[8-11.6]	21.4±5.3[a]	[140-262]	10.4±1.6	[7-13.6]	586.7±88.9[a]	[481-692]
[3-5]	7.6±1.5[a]	[5-9.3]	18.7±7.3[a]	[140-262]	9.7±1.6	[7-13.6]	575.7±68.9[a]	[481-692]
	F=2,728 (P=0.049)		F=5.705 (P=0.017)		F=2.047 (P=0.153)		F=7.971 (P=0,005)	

Values with different letters are significantly different (p < 0.05); Legend: CI: confidence interval; NE: Number Examined; NI: Number Positive; P: prevalence

3.1.2.2.2. Distribution of Egg Density According to the Season

In Table 4, we observe that the average density of flukes varies from 10.03 to 11.88 eggs/gf in liver autopsies, with no significant difference (p>0.05). Similarly, the average egg densities ranged from 232.8 to 289.3 eggs/gf in sedimentation coprology and from 11.8 to 11.84 in Mini-FLOTAC coprology, with no significant difference (p>0.05). However, when examining bile, these densities were significantly higher in the dry season (614.2 eggs/gf) than in the rainy season (812.6 eggs/gf) (p=0.047).

3.1.2.2.3. Distribution of Egg Density According to Breed

The findings in Table 4 show the distribution of egg density according to breed. The average density of flukes ranges from 7.25 to 12.42 eggs/gf for liver autopsies, with no significant difference. However, the average densities of *F. gigantica* eggs range from 238.3 to 277.7 eggs/gf in sedimentation coprology, from 10.12 to 14.44 eggs/gf in coprology by Mini-FLOTAC, and from 553.9 to 769.8 eggs/gf in bile examination. However, these results show no significant difference in this distribution (p> 0.05).

3.1.2.2.4. Distribution of Parasite Densities According to Weight

The results in Table 4 show the distribution of egg density according to weight, including those weighing less than 150 kg, those weighing between 150 and 200 kg, and those weighing more than 200 kg. The average density of flukes varies from 10.33 to 13.63 eggs/gf for liver autopsies, with no significant difference. However, the average egg densities ranged from 237.4 to 450 eggs/gf in sedimentation coprology, from 10.96 to 21 eggs/gf in Mini-FLOTAC coprology, and from 109.3 to 659.4 eggs/gf in bile examination, there was no significant difference in this distribution (P > 0.05) in each test.

3.1.2.2.5. Distribution of Egg Density According to Age

Table 4 shows the distribution of egg density according to age. This includes cattle under 2 years of age, those between 2 and 4 years of age, and those over 4 years of age. The average density of flukes ranged from 6.43 to 11.05 at liver autopsy, with no significant difference. However, the average egg densities varied significantly from 37.5 to 269.5 in sedimentation coprology (F=1.075; ddl= 2 ; p= 0.042), from 1.25 to 12.32 in Mini-FLOTAC coprology (F=1.073; ddl= 2; p= 0.043) and from 550 to 699.8 in bile examination with (F=1.184; ddl= 2; p= 0.032).

3.1.2.2.6. Distribution of Parasite Density According to Sex

The data in Table 4 show the distribution of parasite density in male and female cattle. The average density of flukes varies significantly from 8.04 in males to 11.52 in females for liver autopsies (P=0.049). However, the average egg densities vary from 169.5 to 279.2 in sedimentation coprology, from 7.71 to 13.05 in Mini-FLOTAC coprology, and from 604.8 to 713.6 in bile examination, there were no significant differences in this distribution (P > 0.05) in each test.

3.1.2.2.7. Distribution of Parasite Density According to Body Condition Score

The results in Table 4 show the distribution of egg density according to Body Condition Score (BCN), including those of cattle with BCN= 2 and those with BCN= 3. The average density of flukes varies significantly from 9.8 to 12.7 in liver autopsies with (F=2.728; ddl= 1; p= 0.049). Similarly, the average egg densities varied significantly from 201 to 370 in sedimentation coprology (F=5.705; ddl= 1 ; p= 0.017), and from 586.7 to 912.8 in bile examination (F=7.971; ddl= 1; p= 0.005), with the exception of coprology by Mini-FLOTAC, where it varies from 10.4 to 14.9 without showing any significant difference (P > 0.05).

3.1.3. Determination of the Level of Infestation

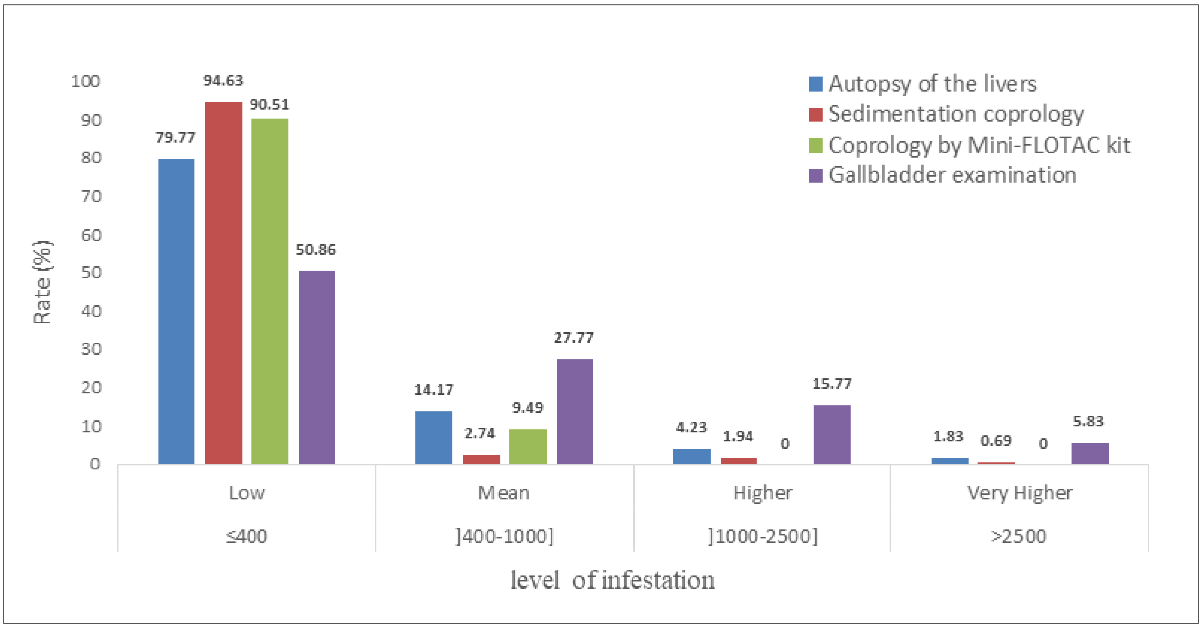

Figure 2: Distribution of cattle infestation levels according to different tests

The results in Figure 2 show the frequencies of infestation levels for each test. This frequency varies from 1.8% to 79.8% for sedimentation coprology, from 9.5% to 90.5% for flotation coprology (Mini-FLOTAC), from 3.2% to 78% for liver autopsy, and from 5.8% to 50.8% for bile examination. It appears that in all tests, low infestation levels are the most frequent. Conclusion: the parasite load in most cattle is low.

3.1.4. Effectiveness of the Mini-Flotac Test

The findings in Table 6 show the variation in sensitivity of the different tests based on the reference test, which is bile examination. The liver autopsy test has the significant highest sensitivity at 69.40%, followed by the flotation/Mini-FLOTAC coprological test at 50.17%, and the sedimentation coprological test which has the lowest sensitivity at 45.90% ($\chi2$= 12.3; ddl= 3; p= 0.001). This study shows that the coprological flotation/Mini-FLOTAC test have a higher sensibility than sedimentation coprological test.

Table 6: Test sensitivity according to the reference test

Examinations	NE	NI	P (%)	Sensibility (%)	P-value
Autopsy of the livers	875	407	46.51	69.45a	0,01
Sedimentation coprology	875	269	30.74	45.90c	
Coprology by Mini-FLOTAC kit	875	294	33.60	50.15b	
Gallbladder examination	875	586	66.97	Reference	

Legend: NE: Number Examined; NI: Number Positive; P: prevalence

3. DISCUSSION

The overall prevalence of fasciolosis varied significantly from 25.25% to 67%. Adult flukes were found in the bile ducts of 46.50% (186/400) of the animals. *F. gigantica* eggs were identified in the bile of 268 of the 400 cattle examined (67%), in the feces of 123/400 of them (30.75%) for sedimentation coprology, and in the feces of 101/400 cattle (25.25%) for flotation/Mini-FLOTAC coprology. Inspection of the livers and coprology tests did not detect all the animals identified as infested, unlike the bile examination. Several methods can be used to estimate the prevalence of the large fluke, but their intrinsic values vary. Simo *et al.,* (2020) compared these various diagnostic methods on 330 cattle for which the prevalence of *F. gigantica* was estimated at 7.3% for liver autopsy, 6.1% for sedimentation coprology, and 33% for bile examination. This is lower than our results but shows a similar trend. Sedimentation coprology and flotation coprology/Mini-FLOTAC did not show any significant differences. Bearing in mind only the prevalence given by the bile examination, the reference test, which was 67% and identified almost all infected cattle, we note that it is lower than that of Abakar (2014), which was 87%, higher than that of Nambafu *et al*., (2015), which was 63.3%, and equal to that of Massamba (2020) in Burkina Faso, which was 67%. However, this prevalence remains high and confirms the findings of Rondelaud *et al*., (2000) that fasciolosis is considered one of the most important helminth infections affecting cattle in tropical regions, with prevalence rates ranging from 25% to 90% in Indonesia, 30% to 90% in Africa, and 25% to 100% in India.

When comparing the different prevalence according to district, we do not notice any significant difference; the district has no real impact on infestation. However, when we vary them according to the season, we notice a significant difference in liver autopsies, which vary from 40.7% in the dry season to 50% in the rainy season. These results show that cattle are more infested in the dry season than in the rainy season. This differs from the results obtained by Siama and Njan-Nlôga (2018) in the Far North of Cameroon, which are 31.49% in the dry season and 23.81% in the rainy season, but similar to those of Iguercha *et al.,* (2021), who obtained a high prevalence (52.3%) in the rainy season. This result may be related to the period of contamination of cows by metacercariae at the end of the dry season. According to Mas-Coma *et al.,* (2005) and, Gragnon *et al*., (2025), when the rate of metacercariae infestation is high at the beginning of the dry season, the rate of parasitosis is higher in the middle of the dry season. Furthermore, the high prevalence of fasciolosis during the rainy season can be linked to climate change and frequent cattle-water-environment interactions (Lamrani *et al*., 2019).

The lower infestation rates in calves than in adults at liver autopsy, sedimentation coprology, Mini-FLOTAC coprology, and bile examination corroborate those obtained by Siama and Njan-Nlôga (2018) in Far North Cameroon, which are (22.09%) in calves, (32.46%) in young animals, and (34.32%) in adults. In contrast, Assogba and Youssao (2001) and Dechasa *et al.* (2012) report that infestation does not vary with age. This difference can be explained by the fact that in the herd, calves are kept around dwellings. However, this result is also justified because in most African countries, the slaughter of calves and heifers is very limited (Youssao, 2013).

The findings that infection does not vary according to sex regardless of the tests differ from those obtained by Siama and Njan-Nlôga (2018) in Far North Cameroon and Raji *et al*., (2010) in Zaria slaughterhouse in Nigeria, who say that females are more infected than males. Ashrafi *et al*., (2014) and Dunn (2003) state that in herds, females outnumber males.

Prevalence based on Body Condition Score (BCS) varies significantly from 42.5% to 55.2% for liver autopsy and from 29.1% to 34.4% for sedimentation coprology. However, it does not vary for the Mini FLOTACT test. These results show that the infestation rate is higher at BCS=2 than at BCS=3. This can be explained by the different symptoms and disorders caused by flukes in animals. According to Sebaa *et al.,* (2015), flukes feed on tissue and liver parenchyma and absorb large amounts of blood. In addition, during the acute phase, symptoms such as fever, digestive disorders, diarrhea, vomiting, nausea, loss of appetite, pain in the right hypochondrium, and hepatomegaly cause significant weight loss (Ayadi *et al*., 1991). The absence of significant differences in the Mini-Flottact test may be due to handling errors or the low sensitivity of the test (Iguercha *et al*., 2021).

The average egg densities in all tests vary only between 614.2 opl in the rainy season and 812.6 opl in the dry season for bile examination. This result is similar to that obtained by Ayadi *et al.,* (1991), who estimated the maximum number of egg passes between December and February and, applying observations from experimental infections, concluded that the infestation period was likely to be between August and September. Similarly, several authors have also noted that these two months were often associated with high *F. hepatica* infestations in France (Andriamanantena *et al.,* 2005) and Tunisia (Ayadi *et al.,* 1991).

The average density of eggs and infestation rates are significantly higher in young and adult animals in sedimentation coprology, Mini-FLOTAC coprology, and bile examination. These results are similar to those reported by Mukatakamba *et al*., (2024) in domestic ruminants in the Democratic Republic of Congo. Older animals are the main reservoirs of the disease (Chartier *et al*., 2000; Merdas, 2015). Thus, according to Dorchies *et al*., (1992) and Merdas (2015), ruminants can develop resistance to the parasite with age, which is linked to repeated infestations, because if ruminants are re-infested with 1,650 metacercariae per animal, only 16% of these larvae are found in the form of adult parasites in the liver. However, this immunity can decrease significantly in older animals (Merdas, 2015).

Parasite loads are significantly higher in females than in males only for liver autopsies. These results are similar to those reported by Siama and Njan Nloga (2018) and Siama *et al.,* (2025) in municipal slaughterhouses in the Far North Region of Cameroon. These results can be explained by the fact that most cows in slaughterhouses are either menopausal or sick (Achukwi *et al*., 2001; Dunn, 2003; Erick *et al*., 2006). In herds, females are more numerous and have greater physiological needs (estrus, conception, milk production, calf nutrition), which forces them to eat more than males and exposes them to a higher risk of parasitic infections (Kaufmann, 1996; Kayoueche, 2009).

The average egg densities were significantly higher in animals with Body Condition Score (BCN)=2 and BCN =3 for sedimentation coprology and bile examination. These results corroborate those of Simo *et al.,* (2020) in cattle from slaughterhouses in western Cameroon. These results show that parasite load varies in line with BCN.

The liver autopsy showed the highest sensitivity at 69.40%, followed by the sedimentation coprological test at 45.89% and the flotation/Mini-FLOTAC coprological test, which showed the lowest sensitivity at 37.68%. This sensitivity was calculated in relation to the reference test, which is bile examination. These results show a significant difference. This study shows that the coprological flotation/Mini-FLOTAC test is less sensitive than the others. This is due to the fact that *F. gigantica* eggs are heavier than the eggs of other trematodes and therefore have difficulty floating and tend to sink despite the high density of the flotation liquid used. Coprology is specific but not very sensitive for screening cases of fasciolosis caused by *F. gigantica*. The examination of a 10 g sample of feces has a sensitivity of over 60%, but such a large amount of feces is never routinely examined because the time required to perform coprology and the low number of eggs detected are demotivating for the technician (Simo *et al*., 2020). By increasing the weight of the sample examined, sensitivity increases significantly. Braun *et al.,* (1995) found that sensitivity varied depending on the amount of feces examined: for 4 g, sensitivity was 43%, but it reached 64% for 10 g. These results are consistent with those of Rapsch *et al*., (2006).

The conditions of the study did not allow for an increase in the size of the stool samples examined. The prevalence estimated using coproscopy [Mini-FLOTAC (25.25%) and sedimentation (30.75%)] was lower than that estimated by bile examination (67%); the sensitivity of coproscopy was 37.68% for Mini-FLOTAC and 45.89% for sedimentation compared to bile examination (reference test). However, it was higher than those obtained by Youssao and Assogba (2002) in the Niger River valley (11%) and by Achukwi *et al.,* (2001) at Ngaoundere in Cameroon (8%).

Liver autopsy in slaughterhouses was more sensitive than coproscopy (prevalence of 46.50% and sensitivity of 69.40%) and was specific. European regulations 854/2004 and 1244/2007 only recommend visual inspection of meat, i.e., without the mandatory routine techniques of palpation and incision of viscera or carcasses used in this study. Under these conditions, a number of cases of fasciolosis would escape the inspectors, but this risk would be negligible to very low (Theodoropoulos *et al*., 2002). The results show the inadequacy of liver autopsy and the value of searching for eggs in bile. In practical terms, this technique is simple to implement and provides reliable results even when laboratory working conditions are difficult. During this study, the ELISA test was not performed due to a lack of financial resources. This cannot have had a significant impact on the results, as the sensitivity of the tests is slightly lower than that of bile examination (Rapsch *et al*., 2006). The ELISA test is an indicator of the presence or previous occurrence of a parasitic infestation. It is also possible that seropositivity indicates contact with the parasite in the preceding months, but that due to an insufficient number of metacercariae (the infesting parasitic stage) ingested by the animal, these contacts did not result in infestation. It is also possible that seropositivity indicates contact with the parasite in the preceding months, but that due to an insufficient number of metacercariae (the parasitic infestation stage) ingested by the animal, these contacts do not result in infestation, i.e., the growth of adult flukes in the bile ducts. These are then false positives. However, given the low receptivity of cattle, less than 10% of metacercariae complete their development in this species, whereas this percentage is higher (30–50%) in sheep, the most receptive species (Mekroud *et al*., 2006). Despite this, testing for specific antibodies in blood and milk is considered a sensitive and specific tool for screening for the large fluke (Tliba, 2001).

CONCLUSION

This study shows that it is possible to demonstrate the presence of fasciolosis in animals with the Mini-FLOTACT Kit, because we obtained overall prevalence's of 33.58% for coprology using the Mini-FLOTAC Kit against 30.75% for coprology by sedimentation, 46.50% for the prevalence by liver orthoptics, and 67% for the bile examination. The evaluation of prevalence, parasite densities and infestation levels according to intrinsic factors is comparable to those of other tests, except that the parasite loads are lower than that of coprology by sedimentation. This test, which has an efficiency of 50.17% compared to the reference test, is less sensitive than that of the liver autopsy (69.45%), but more sensitive than coprology by sedimentation (45.9%). It can therefore be recommended for the rapid detection of fasciolosis in herds and country farms while waiting to complete with serological analyzes in the laboratory. In this perspective, it would be wise to test its effectiveness in screening other helminthiases of medical and veterinary interest.

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