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Bovine fasciolosis in two Algerian slaughterhouses: Prevalence and assessment of liver suitability for human consumption

Lynda Mezali[1*], Siham Nouichi[2], Saliha Bouabba[1], Kenza Hettak[1], Nadia Negab[1], Rachid Kaddour[1], Amina Dahmane[1]

[1]*Ecole nationale supérieure vétérinaire Rabie Bouchama, Algiers, Algeria.*
[2]*Centre universitaire Abdelhafidh Boussouf, Mila, Algeria.*

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***Correspondence:**

Corresponding author: Lynda Mezali
E-mail address: l.mezali@ensv.dz

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ABSTRACT

Fasciolosis, a hepatobiliary distomatosis affecting domestic ruminants and incidentally humans, poses a threat to both animal and public health, leading to substantial economic losses. This study aimed to determine the prevalence of fasciolosis in cattle slaughtered in Tizi Ouzou, Algeria, and to describe the morphological and histological changes in the liver. A total of 376 bovine carcasses slaughtered between July 2018 and April 2019 underwent postmortem inspection, and corresponding livers were investigated for fasciolosis according to conventional meat inspection procedures. Twenty-six livers, found to be affected with fasciolosis, were sampled at five different sites for histopathological analysis using both hematoxylin-eosin (H&E) and Masson's trichrome stain methods. The overall prevalence was 6.9% (CI 4.4-9.5%). Gross changes related to bovine fasciolosis, including size, color, and consistency abnormalities as well as the worms' migratory path and distomian cholangitis, were not observed in the majority of the affected livers. Additionally, the infestation degree was low in 56.5% of the fasciolosis-affected livers. In contrast, numerous histopathological damages, including infiltrations, degeneration, necrosis, and fibrosis, were revealed in hepatic parenchyma, connective septa, portal region, and bile ducts throughout all the sampled liver tissues (P> 0.05). Hepatic involvement observed in both the acinus and portal lobule throughout their respective zones was quantified with a degree ranging from 25% to 100% (P> 0.05). Fasciolosis, a common disease in cattle slaughtered in Tizi Ouzou, induces irreversible hepatic tissue damage, thereby compromising the organoleptic and nutritional value of the liver, making it unfit for human consumption.

Introduction

Fasciolosis, more commonly known as liver fluke infection, is caused by two species of trematode helminths: *Fasciola hepatica* in temperate climates and *Fasciola gigantica* in tropical areas. This zoonotic disease is a significant veterinary and public health concern worldwide that affects both humans and animals. Considered an emerging disease due to the influences of climate and global changes, an estimated 2.4 million people in over 75 countries are annually *Fasciola* spp.-infected, with 180 million at risk of infection. The infection exhibits diverse epidemiological patterns, adapting to various ecological niches (Torgerson and Macpherson, 2011; Elshraway and Mahmoud, 2017; Jaja *et al.*, 2017; WHO, 2020; Bentounsi and Cabaret, 2023). The life cycle of *Fasciola* is complex, involving infested animals that perpetuate the infection in the environment and intermediate hosts, specifically the *Lymnaea* snail *Galba truncatula*. Infection occurs through the ingestion of metacercariae, either via contaminated aquatic vegetation or water (Torgerson and Macpherso, 2011; Arafa *et al.*, 2017; WHO, 2020), followed by migration within the hepatic parenchyma to the bile ducts (Bentounsi and Cabaret, 2023). Human infection may also occur through the ingestion of immature flukes or eggs present in contaminated food. Risk factors, such as age (young) and seasonal conditions (grazing), contribute to the complexity of fasciolosis epidemiology (Torgerson and Macpherson, 2011; Jaja *et al.*, 2017; WHO, 2020).

Hepatic fasciolosis affects over 600 million animals globally (Jaja *et al.*, 2017) and contributes to morbidity and mortality in a wide range of mammals, including domestic ruminants such as cattle (Bentounsi and Cabaret, 2023). It severely impacts livestock industries, causing substantial economic losses by reducing weight gain, meat yield, fertility and calf birth weight, decreasing milk and wool production with reduced quality, and leading to the condemnation of emaciated carcasses, especially those with affected livers (Torgerson and Macpherson, 2011, Bentounsi and Cabaret, 2023).

Data on the prevalence of fasciolosis is well documented in Northern Algeria, both within cattle breeding farms and slaughterhouses (Mekroud *et al.*, 2004; Aissi *et al.*, 2009; Boucheikhchoukh *et al.*, 2012; Merdas Ferhati *et al.*, 2014; Ouchene-Khelifi *et al.*, 2018; Ayad *et al.*, 2019; Chaouadi *et al.*, 2019; Chougar *et al.*, 2019; Meguini *et al.*, 2021). There are various diagnostic methods for the accurate assessment and control of *Fasciola* infection. In live animals, coproscopy has limitations due to irregular egg shedding. However, immunological techniques, including ELISA on serum or milk as well as ELISA kits for fecal detection offer better sensitivity for both individual and group screenings (Bentounsi and Cabaret, 2023). At the slaughterhouse level, postmortem inspection is legally required, which involves visual observation, palpation, and incision of both the liver parenchyma and bile ducts. This procedure is essential for detecting and removing *Fasciola* spp.-affected livers. It helps prevent contaminated meat from entering the food chain, thereby reducing the risk of human infection. Additionally, it provides valuable data on the prevalence of fasciolosis, which aids in implementing control measures and interventions. The examination of bile appears to be the most sensitive method, achieving 80% of sensitivity and 97% of specificity, as reported by Chaouadi *et al.* (2019). Nevertheless, in a previous study (unpublished results), microscopic bile examination did not detect *Fasciola* eggs in two fasciolosis-infested livers.

Hepatic fasciolosis involves the migration of metacercariae through the digestive system and liver, resulting particularly in inflammation and fibrosis. The severity of these damages depends on the intensity and duration of the infection (Euzeby, 1998). To mitigate economic losses

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in certain Algerian slaughterhouses, the fasciolosis-affected liver is systematically and randomly trimmed at the hepatic hilum region instead of being condemned if a gross examination reveals no visible changes. Consequently, histopathological analysis may provide insights into the extent of damage, inflammatory response, and disease progression. It serves as a valuable tool for achieving accurate diagnosis.

To the best of our knowledge, there is a lack of published data on the prevalence of bovine fasciolosis among cattle slaughtered in Tizi Ouzou, a region renowned for its prominent status in cattle farming. Furthermore, there is a dearth of information regarding histopathological assessments of liver quality in Algeria (Taibi *et al*., 2019). In this study, our objective was to determine the prevalence of fasciolosis in cattle slaughtered in Tizi Ouzou and evaluate the intensity of fluke infestation. We described the morphological and histopathological changes in hepatic tissues and assessed liver quality by quantifying the degree of histological involvement.

Materials and methods

Study area

This study was conducted at two low-throughput slaughterhouses in Tizi Ouzou, a coastal region in North-Central Algeria well-known for its cattle farming. The two slaughterhouses of Azazga and Tamda are private meat processing facilities located in the municipalities of Azazga and Ouaguenoun, respectively. They operate every day of the week except Fridays and Saturdays. The frequency of slaughtering varies depending on the season and occasions, with a higher pace during the summer, family holidays, and Ramadan.

Postmortem inspection and sampling

A total of 376 slaughtered cattle between July 2018 and April 2019 were screened to assess the prevalence of fasciolosis, without distinguishing by breed, gender, or age. However, in compliance with Algerian regulations, the slaughter of bovine females is prohibited before the age of 8 years for cross breeds and 5 years for local breeds. Consequently, cattle males are the most slaughtered.

After the completion of dressing, the outer surface of the carcasses, as well as the thoracic and abdominal cavities, were visually examined for any abnormalities or pathological lesions. All internal organs also underwent thorough veterinary inspection according to conventional meat inspection procedures. Each liver, which remained attached to its corresponding carcass, was examined through visual observation and palpation of both the visceral and diaphragmatic surfaces. Furthermore, two incisions were made on the visceral surface. The first incision, long and superficial, was made on the left lobe, while the second incision, short and deep, was performed at the base of the Spiegel's (caudal) lobe. The main purpose of these incisions, as well as the opening of the common bile duct, was to detect the adult (mature) worm of liver flukes. Subsequently, the hepatic (portal) and hepato-pancreatic lymph nodes were visually examined and incised. The bovine livers showing signs of fasciolosis (with or without distomian cholangitis; distomian cholangitis refers to inflammation of the bile ducts with the presence of the adult worms) were sampled from five different selected sites: the right lobe (RL), the left lobe (LL), the Spiegel's lobe (SL), the hepatic hilum region (HH), and the deep parenchyma in direct contact with the hepatic hilum region (DP). A total of 130 samples were collected, preserved in 10% formalin solution, and transported to the histopathology laboratory at ENSV. In addition, flukes found in *Fasciola* spp.-infested livers, were collected, counted, and preserved in 70% ethanol.

Preparation of histological slides for liver tissues

In brief, the affected liver specimens are initially cut into small fragments (10x10x5 mm) and placed in pre-identified cassettes. The prepared cassettes undergo a series of steps, including washing with distilled water, dehydration by immersing in two consecutive baths of ethanol with ascending concentrations (85 and 90%) and two baths of methanol, clearing with toluene, and impregnation and blocking in liquid paraffin.

The paraffin-embedded fragments are then sectioned into 5 µm thick slices using a rotary microtome. The resulting ribbons are flattened in a water bath, picked up with glass slides, and dried in an oven. Two different staining techniques are performed, hematoxylin-eosin (H&E) stain and Masson's trichrome stain, resulting in the preparation of two slides for each of the 130 samples. H&E staining involves the application of hematoxylin and eosin dyes, while Masson's trichrome staining employs a multi-step process with multiple dyes. After staining, the slides are dewaxed, rehydrated with descending concentrations of ethanol (100, 95, and 70%), and washed. Finally, the slides are mounted with synthetic resin and allowed to dry under a coverslip (Okoye *et al*., 2015). All histological slides are examined using a light microscope at various magnifications (4x, 10x, and 40x).

Lesions in the parenchyma, connective septa, and portal region were described for the five sampled sites, while lesions associated with the bile ducts were only described for the hepatic hilum region.

Evaluation of Fasciola spp. infestation degree in the hepatic tissue

The degree or intensity of infestation in fluke-infested livers was conducted based on the detected adult worm's count. The infestation was categorized as either low or moderate when the number of flukes ranged from 1 to 10 or 11 to 20, respectively. Infestations with more than 20 flukes were classified as massive.

Quantification of histological involvement in the hepatic parenchyma

The zoning method was used with particular emphasis on degenerative and necrotic lesions. The classification scheme of this method was applied based on two descriptions: a pathological description focused on the hepatic acinus, delineating three zones (I: the furthest zone from the centrolobular vein, II: intermediate zone between zones I and III, and III: the closest zone to the centrolobular vein), and a physiological description specific to the hepatic portal lobule, delineating two zones (A: the closest zone to the portal region, and B: the furthest zone from the portal region). To enable quantitative assessment, each histological slide was divided into five observation fields, allowing the calculation of the involvement degree for each field.

Data analysis

A database was created using Microsoft Office Excel software to manage data related to sampled livers, postmortem lesions, and zoning classification results. Statistical significance was evaluated with a 95% confidence interval, and statistical comparisons were performed using IBM SPSS Statistics software version 21.0.0.0 (2012) to evaluate the rates of macroscopic and microscopic lesions, as well as the rates of histological estimation of hepatic damage effects, between the sampled livers and the 5 selected hepatic sites. The threshold for statistical significance was set at a P-value of 0.05.

Results

Prevalence of bovine fasciolosis

Out of the 376 examined bovine carcasses (170 at Azazga slaughterhouse and 206 at Tamda slaughterhouse), 26 were found to have fasciolosis-affected livers. Consequently, the overall prevalence of fasciolosis is calculated as 6.9% (CI 4.4-9.5%), with Azazga and Tamda reporting specific prevalence rates of 8.8% (CI 4.6-13.1%) and 5.3% (CI 2.3-8.4%), respectively (Table 1).

Table 1. Prevalence of hepatic fasciolosis in cattle slaughtered in two slaughterhouses in Tizi Ouzou.

Slaughterhouse	N	Positive N (%)	95% CI
Azazga	170	15 (8.8 %)	[4.6 - 13.1]
Tamda	206	11 (5.3 %)	[2.3 - 8.4]
Total	376	26 (6.9 %)	[4.4 - 9.5]

N: Number of inspected bovine livers; 95% CI: 95% confidence interval.

Macroscopic examination and infestation intensity of the affected livers

Postmortem examination revealed characteristic macroscopic changes and lesions in certain fasciolosis-affected bovine livers, as shown in Fig. 1.

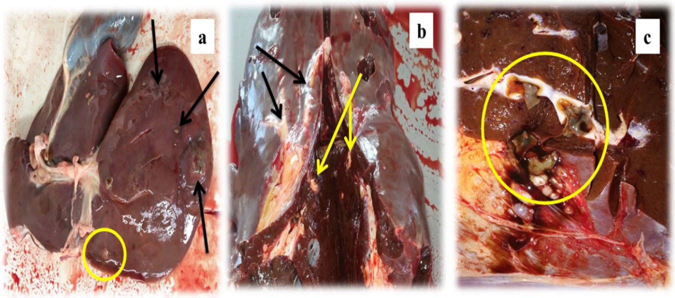

Fig. 1. Characteristic macroscopic changes and lesions on the diaphragmatic surface associated with bovine fasciolosis: (a: black arrow) migratory paths of worms, (a: yellow circle) formation of abscess, distomian cholangitis characterized by (b: black arrows) appeared bile ducts on the liver surface, (b: yellow arrows) thickening and calcification of the bile duct wall with deposition of hemosiderin sediment, and (c: yellow circle) presence of mature worms within the bile ducts' lumen.

The macroscopic characteristics of the hepatic parenchyma and bile ducts are reported in Table 2. Among the 26 sampled livers, 76.9%, 61.5%, and 73.1% displayed a normal size, color, and consistency of the parenchyma, respectively. The bile ducts were visible on the surface of 69.2% of the sampled livers, while 88.5% (n= 23) of them were fluke infested.

The infestation degree by *Fasciola* spp. was generally low, with the number of counted flukes ranging from 1 to 10 in over half (56.5%) of the sampled bovine livers (Table 3).

Histopathological analysis of liver tissues

The examination of 260 histological slides revealed numerous lesions that have damaged the parenchyma, connective septa, portal region, and bile ducts throughout the entire liver. No difference (P > 0.05) was observed between the hepatic hilum region (HH) and the other four sampled sites (RL, LL, SP, DP) (Table 4 and Fig. 2).

In Table 5, the results of the quantification of the degree of histological involvement for the hepatic parenchyma are presented. The involvement was observed in both the acinus and portal lobule in their respective zones. Across all sampled livers, varying degrees of involvement were recorded, with the predominant range being from 50% to 75% for both the acinus and portal lobule.

No significant difference (P> 0.05) was observed in the involvement of the hepatic parenchyma between zones or sampling sites.

Table 2. Macroscopic examination of the fasciolosis-affected bovine livers.

Examined liver part	N	%	95% CI
Hepatic parenchyma			
Size			
Normal	20	76.9	[60.7 - 93.1]
Atrophy	2	7.7	[0.0 - 17.9]
Hepatomegaly	4	15.4	[1.5 - 29.3]
Color			
Normal	16	61.5	[42.8 - 80.2]
Petechiae	7	26.9	[9.9 - 44.0]
Hemorrhage	3	11.5	[0.0 - 23.8]
Consistency			
Normal	19	73.1	[56.0 - 90.1]
Friable	3	11.5	[0.0 - 23.8]
Rigid	4	15.4	[1.5 - 29.3]
Bile ducts			
Appeared on the liver surface			
Yes	18	69.2	[51.5 - 87.0]
No	8	30.8	[13.0 - 48.5]
Adult worms of liver fluke			
Absence	3	11.5	[0.0 - 23.8]
Presence	23	88.5	[7.2 - 100.0]

N: Number of inspected bovine livers; 95% CI: 95% confidence interval.

Table 3. Infestation degree of the sampled bovine livers by *Fasciola* spp.

Infestation degree	Classification range	N	%	95% CI
Low	1 to 10	13	56.5	[36.3 - 76.8]
Moderate	11 to 20	7	30.4	[11.6 - 49.2]
Massive	≥ 21	3	13	[0.0 - 26.8]

N: Number of flukes-infested livers.

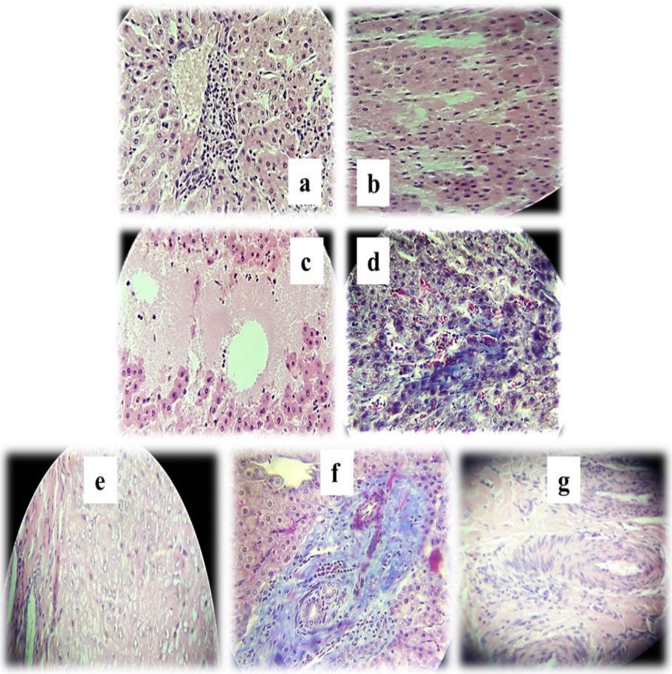

Fig. 2. Light microscopy examination of H&E and Masson's trichrome stained histological slides for hepatic tissues revealed damage effects by characteristic lesions in the parenchyma: (a) diffuse infiltration, (b) degeneration, (c) necrosis, (d) fibrosis; in (e) the connective septa (diffuse and focal infiltrations, degeneration, necrosis, fibrosis and thickening); in (f) the portal region (diffuse infiltration, fibrosis, vascularitis, edema, and thickening, ulceration or desquamation of the bile ducts); and in (g) the main bile ducts (diffuse and focal infiltrations, fibrosis, vascularitis, thickening, ulceration, and desquamation).

Table 4. Histopathological changes observed in fasciolosis-affected livers based on sampling site.

Lesion	Sampling site (*P*> 0.05)									
	RL		LL		SP		HH		DP	
	N	%	N	%	N	%	N	%	N	%
Parenchyma										
Diffuse Infiltration	26	100	26	100	26	100	26	100	26	100
Degeneration	26	100	26	100	26	100	26	100	26	100
Necrosis	26	100	26	100	26	100	26	100	26	100
Fibrosis	11	42.3	12	46.2	17	65.4	9	34.6	12	46.2
Connective septa										
Diffuse infiltration	26	100	24	92.3	25	96.2	26	100	26	100
Focal infiltration	0	0	1	3.8	1	3.8	0	0	0	0
Degeneration	26	100	22	84.6	22	84.6	24	92.3	22	84.6
Necrosis	25	96.2	23	88.5	21	80.8	23	88.5	22	84.6
Fibrosis	15	57.7	17	65.4	18	69.2	7	26.9	11	42.3
Thickening	16	61.5	22	84.6	16	61.5	15	57.7	17	65.4
Portal region										
Diffuse Infiltration	26	100	26	100	26	100	25	96.2	26	100
Fibrosis	20	76.9	18	69.2	22	84.6	25	96.2	19	73.1
Vascularitis	12	46.2	13	50	12	46.2	6	23.1	13	50
Edema	0	0	0	0	1	3.8	0	0	1	3.8
Thickening[a]	23	88.5	23	88.5	20	76.9	24	92.3	19	73.1
Ulceration[a]	3	11.5	2	7.7	2	7.7	1	3.8	0	0
Desquamation[a]	0	0	0	0	0	0	0	0	2	7.7
Bile ducts										
Diffuse infiltration	-	-	-	-	-	-	18	69.2	-	-
Focal Infiltration	-	-	-	-	-	-	2	7.7	-	-
Fibrosis	-	-	-	-	-	-	24	92.3	-	-
Vascularitis	-	-	-	-	-	-	2	7.7	-	-
Thickening	-	-	-	-	-	-	21	80.8	-	-
Ulceration	-	-	-	-	-	-	15	57.7	-	-
Desquamation	-	-	-	-	-	-	8	30.8	-	-

[a]Lesions associated with the bile ducts in the portal region.

N: Number of the sampled livers; RL: Right lobe; LL: Left lobe; SL: Spiegel's lobe; HH: Hepatic hilum region; DP: Deep parenchyma in direct contact with the hepatic hilum region.

Table 5. Estimation of involvement degree in hepatic acinus (I, II, and III) and portal lobule (A and B) according to the observation zone and sampling site.

Sampling site Zone	RL N					LL N					SL N					HH N					DP N				
	I	II	III	A	B	I	II	III	A	B	I	II	III	A	B	I	II	III	A	B	I	II	III	A	B
25% [a]	6	0	28	22	6	5	0	30	23	10	15	0	28	31	13	15	0	32	28	16	8	0	32	24	12
50% [a]	55	71	67	73	52	61	79	62	67	54	59	93	68	73	63	61	69	65	67	62	62	77	64	67	52
75% [a]	64	41	32	31	67	64	44	35	37	64	55	25	33	25	53	48	31	28	29	42	59	38	34	39	63
100% [a]	3	16	1	2	3	0	7	3	3	2	1	12	1	1	1	1	25	0	1	5	1	15	0	0	3
ND	2	2	2	2	2	0	0	0	0	0	0	0	0	0	0	5	5	5	5	5	0	0	0	0	0

N: Number of the examined microscopic fields (Total= 130; 5 fields per slide).
Observation zones (P> 0.05):
I: Periportal region located adjacent to the portal triad; II: midzonal region located between zone I and zone III within the hepatic lobule; III: pericentral region located adjacent to the centrolobular vein, and the farthest zone from the portal triad.
A: the closest zone to the portal region; B: the furthest zone from the portal region.
[a]: Degree of histopathological involvement of the hepatic parenchyma. ND: degree not determined.
Sampling site (P> 0.05); RL: Right lobe; LL: Left lobe; SL: Spiegel's lobe; HH: Hepatic hilum region; DP: Deep parenchyma in direct contact with the hepatic hilum region.

Discussion

Postmortem examination of the liver serves as a direct diagnostic method for determining bovine fasciolosis. Using this approach, the overall prevalence of hepatic fasciolosis in cattle slaughtered at two slaughterhouses in Tizi Ouzou was estimated to be 6.9%, with 8.8% in Azazga and 5.3% in Tamda (Table 1). This result was in close agreement with our previous findings at the same slaughterhouses between 2017 and 2018 (6.7%, unpublished results), as well as in Northern Algeria (5.1%) as reported by Chougar *et al.* (2019). Noteworthy lower prevalence results were reported from other slaughterhouses across different regions of Algeria: 2.9% in the Mitidja region (Chaouadi *et al.*, 2019), 1.7% in Ouargla (Ouchene-Khelifi *et al.*, 2018), and 2.83% in Bejaia (Ayad *et al.*, 2019). In contrast, higher prevalence rates of bovine fasciolosis have been documented by Mekroud *et al* (2004) in Constantine (9.1%) and Jijel (27%) slaughterhouses, Boucheikhchoukh *et al.* (2012: 52.4%), Ouchene-Khelifi *et al.* (2018: 26.7%) in El Tarf slaughterhouses, and Meguini *et al.* (2021: 12.3%) in Souk Ahras slaughterhouses. Bovine fasciolosis appears to be widespread in Algeria. The absence of a control strategy, combined with the Mediterranean climate, may contribute to the resurgence and dispersion of fasciolosis populations, according to Chougar *et al.* (2019).

In Africa, the prevalence of *Fasciola* spp. infestation in slaughtered cattle ranged from 11.1% (Arafa *et al*., 2017) to 30.9% % (Elshraway and Mahmoud, 2017) in Egypt, was 12.6% in Tunisia (Hamed *et al*., 2014), and 83.6% in South-Eastern Ethiopia (Bayou and Geda, 2018). Worldwide, different proportions of bovine fasciolosis were noted: 2.2% in Iran (Khanjari *et al*., 2010), 5.45% in Turkey (Kara *et al*., 2009), 23.68% in Ireland (Byrne *et al*., 2016), and 29.51% in Brazil (Dutra *et al*., 2010).

The disparities in prevalence within the same country and between countries could be influenced by numerous factors, such as climate changes and environment, rainfall patterns, availability or not of intermediate hosts, animal origin, contaminated livestock feed/water and other breeding conditions (Mekroud *et al*., 2004; Chaouadi *et al*., 2019; Bentounsi and Cabaret, 2023), as well as the sample size, period and duration of the studies.

According to conventional meat inspection procedures and in compliance with Algerian regulations, bovine livers found to be affected by fasciolosis should be condemned to ensure public health, despite the significant economic impact. The visual inspection and palpation of the 26 sampled livers revealed that the majority exhibited normal size (76.9%), color (61.5%), and consistency (73.1%). Gross changes observed in only a few livers (Table 2), are characteristic signs of fasciolosis (Adrien *et al*., 2013) that have been reported previously, including hepatomegaly and brownish color with petechiae in 61.5% and 57.7% of the affected bovine livers, respectively (Unpublished results). The morphology of fasciolosis-affected livers may be influenced by various factors, including the animal's sensitivity, associated with its overall health status and individual resistance, the chronicity of the disease, the degree of infestation, and the age of the *Fasciola* flukes (Bentounsi and Cabaret, 2023).

Regarding the bile ducts, hardness and thickening were observed, making only 18 out of the 26 sampled livers, visible on the visceral surface. Upon incision of the common bile duct, the adult worms were detected in 23 of them. Our findings, supported by those of Simo *et al*. (2020), suggest that the presence of flukes is not necessarily correlated with the bile ducts visibility, highlighting the importance of performing mandatory investigative incisions. In addition, the lumen of the bile ducts was filled with sediment containing hemosiderin, a consequence of elevated iron concentration. These distinctive damages, illustrated in Fig. 1, are known distomian cholangitis. They are directly attributed to the aggressive behavior of the parasites, as the bile ducts are the final habitat for mature flukes (Dorchies *et al*., 2012; Adrien *et al*., 2013; Okoye *et al*., 2015; Simo *et al*., 2020). Notably, 11.5% (n= 3) of the livers exhibited non-distomian cholangitis. This could likely be explained by the elimination of *Fasciola* flukes after the establishment of a delayed immune defense mechanism in the bile ducts, typically occurring six months after infestation (Gourreau and Schelcher, 2011), or as a result of appropriate flukicide treatments (Chauvin and Boulard, 1992).

On the other hand, the infestation degree varied among the 23 bovine livers which were found to be infested by *Fasciola* flukes. The majority of livers (n= 13) showed a commonly low infestation degree, with the count of adult worms ranging from 1 to 10, while 7 and 3 livers exhibited a moderate and massive infestation degree, respectively (Table 3). These findings suggest that the observed differences in infestation intensity may be due to variations in the number of ingested metacercariae by each animal, which can be influenced by the climatic conditions of the breeding region (Bentounsi and Cabaret, 2023).

In the present study, the histopathological analysis focused on two distinct parts of the fasciolosis-affected liver: the hepatic hilum region (HH), which is always discarded, and the remaining part of the liver (including RL, LL, SP, and DP), which is deemed suitable for human consumption in certain slaughterhouses. The examination of histological slides performed by using two staining methods (H&E and Masson trichrome's stain), enabled the identification of various pathological features within different structural components of the liver (Table 4), but without difference between the sampling sites (P> 0.05).

In the hepatic parenchyma, diffuse infiltration, degeneration, and necrosis were concurrently observed in 25 out of the 26 sampled livers (Fig. 2a, 2b and 2c). The involvement of hepatocytes is attributed to the trauma caused by the digestion of host components during the migration of immature worms. These parasites, exhibiting histophagous behavior, release proteases (McGavin *et al*., 2001) to trigger inflammatory reactions (hemorrhage). This process involves the aggregation of lymphocytes and eosinophils, resulting in infiltrations (Doy and Hughes, 1984; Wolf ,1999; Tliba *et al*., 2000; Okoye *et al*., 2015). In addition, the liver invasion by migrating immature liver fluke creates anaerobic conditions conducive to bacterial germination and proliferation, leading to hepatocellular necrosis and abscess formation (Salmo *et al*., 2014). Subsequently, healthy liver tissue is gradually replaced by fibrous scar tissue (Dawes, 1970). Our findings align with those reported in Algeria by Merdas Ferhati *et al*. (2015), who compared livers of healthy animals with those of infested animals, and by Taibi *et al*. (2019) who evaluated the hepatocellular integrity for one liver, suggesting a chronic bovine fasciolosis.

As for the connective septa, degeneration was observed in all the sampled livers, while necrosis, fibrosis, and thickening were present in 25 of them (Fig. 2e). These lesion features are a direct consequence of the involvement of the hepatic parenchyma.

In the portal region, numerous lesions were observed (Fig. 2f). Diffuse infiltration, fibrosis, and bile duct evolvement, particularly thickening, were present in all livers, with the majority of them (22 out of 26) presenting vascular inflammation. The frequency of lesions in the portal region can be attributed to the hematophagous diet of mature worms (Bentounsi and Cabaret, 2023). The parasites can erode the bile ducts using their tegumental spines, resulting in hemorrhages, epithelial thickening, and ultimately leading to hyperplasia. These findings align with a study conducted by Barnouin *et al*. (1981), which compared between healthy livers, livers with distomian cholangitis but no lesions, and livers with generalized lesions, demonstrating the presence of inflammatory and necrotic lesions in both the portal region and parenchyma. Likewise, Mebanga (1993) reported similar lesions in 58 samples of fasciolosis-affected bovine livers.

The lesions affecting the bile ducts were primarily observed in the samples taken from the hepatic hilum region, where the main and secondary bile ducts converge. In this important region of the liver, a considerable number of lesions were also noted (Fig. 2g). Specifically, notable pericanalicular fibrosis was observed in 24 of the sampled livers, and epithelial thickening was noted in 21 of them. These two particular lesions, characteristic of distomian cholangitis, are considered the most significant pathological features associated with fasciolosis in cattle, as stated by Dawes (1970).

The degree of histopathological involvement was quantitatively assessed in the hepatic acinus (zones I, II, and III) and portal lobule (zones A and B) based on the observation zone and sampling site. Microscopic examination revealed the presence of degeneration and necrosis in all 26 sampled livers at the 5 sampling sites, with varying degrees ranging from 25% to 100% (Table 5). Data analysis showed no difference in the degree of involvement between zones I, II, and III (P> 0.05), and between zones A and B (P> 0.05). However, It is important to highlight that the involvement of zone I, the closest zone to the portal triad and the first zone encountered by blood flow entering the liver lobule, results in irreversible histopathological damage. Consequently, both zone II and, notably, zone III, the furthest from the portal triad and receiving blood that has passed through the two preceding zones, also experience damage.

The involvement of the hepatic acinus is attributed to disturbances or even cessation of blood circulation in the sinusoids, essential for maintaining acinus integrity. This disruption may result from cellular infiltrations, edema, and the fibrosis process, leading to compression and occlusion of blood vessels (Dorchies *et al*., 2012). On the other hand, the integrity of the portal lobule relies on proper bile drainage, where a group of adjacent lobules is drained by a common bile duct. In the case of *Fasciola* infestation, the parasite causes calcification and obstruction of the bile ducts, disrupting bile circulation and allowing the parasite to ascend towards secondary ducts, affecting the lobules (Wolf, 1999).

Conclusion

The current study confirmed the presence of bovine fasciolosis in two slaughterhouses in Algeria, with a prevalence of 6.9%. Although the macroscopic examination revealed regular size, color, and consistency in the majority of the sampled livers, as well as 30.8% (n=8) did not exhibit visible bile ducts on the surface, and more than half (n=13) showed a low degree of infestation, the histopathological analysis revealed numerous and extensive damages to hepatic parenchyma, connective septa, portal region, and bile ducts, regardless the sampling site. Furthermore, quantitative assessment of histopathological involvement for degeneration and necrosis recorded degrees ranging from 25% to 100% in the 26 sampled liver tissues, including both the naturally trimmed hepatic hilum region and the liver part that is sometimes deemed suitable for human consumption.

This study provides valuable insights into the harmful impact of fasciolosis infections on public health and the compromised organoleptic and nutritional quality of the liver when consumed by humans. The findings highlight the need to avoid systematic and random trimming of fasciolosis-affected livers useful in certain slaughterhouses, even if they appear to have normal morphology. Hence, veterinary public health and environmental measures, including early detection of fasciolosis at the farm level, strategic anthelmintic treatment of livestock, strict enforcement of husbandry-human separation, and effective eradication of intermediate hosts, are indispensable components to prevent potential health risks and mitigate significant economic losses associated with liver condemnation.

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Conflict of interest

The authors declare that they have no competing interests.

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