



# Evaluation of *Fasciola hepatica* Infections in Cattle in Northeastern Algeria and the Effects on Both Enzyme and Hepatic Damage, Confirmed by Scanning Electron Microscopy

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## Abstract

**Purpose** The aim of our study is to establish the presence of *Fasciola hepatica* on farms in northeastern Algeria.

**Methods** 143 blood and coprological samples of 15 males and 128 females of different breeds and ages were analysed.

**Results** Our study indicates a heterogeneous level of the anti-f2 antibodies to *Fasciola hepatica* in response to the infection. The overall seroprevalence was about 22.37%, and 9 out of 13 investigated farms were infected, with rates varying from 5.88% to 70%. To explain the intrinsic variability of the infection, we identified age, sex and breed as potential risk factors. Based on this, we collected information about their relevance. There was a significant difference for age ( $p = 0.018$ ) and coprology ( $p < 0.0001$ ). Independently, sex and breed had no impact on the infection, although males were more infected (27%) than females (22%). Of the five investigated breeds, Holstein cattle (31.11%) were most affected, followed by Montbeliard (20.27%) and crossed breed (13.64%). Multivariate comparisons showed that the presence of faecal eggs reflects the active infectious status of cattle ( $p < 0.0001$ ), while age ( $p = 0.011$ ) and sex ( $p = 0.040$ ) significantly impact the chance of acquiring the infection. To evaluate liver parenchyma integrity and its functionality, hepatic enzymes were examined and showed relatively low levels of aminotransferases, excluding cytolysis. Although sensitive to distomatosis,  $\gamma$ GT and PAL values were inconsistent with the infection rate. The relatively high levels of proteins and albumin eliminate hepatic insufficiency.

**Conclusion** Our results suggest a chronic fasciolosis, confirmed by histology and SEM.

## Article Highlights

- The northeast of Algeria is classified as an area with fasciolosis.
- Confirmation of enzymatic data by chronology of histological events.
- Invalidation of the relationship between anti-f2 antibodies to *Fasciola hepatica* and the Gamma GT levels, identified as one of the markers of the canalicular enzymes.
- Superposition of macroscopic observations to SEM / EDX images showing calcified structures referring to histological data.

**Keywords** Cattle · Elisa · *Fasciola hepatica* · Liver enzyme · Liver injuries

## Abbreviations

$\gamma$ GT Gamma glutamyl transferase  
PAL Alkaline phosphatase  
SEM Scanning electron microscopy

## Introduction

Of the many parasitic infections that occur in northern Africa, fasciolosis appears to be one of the most common zoonotic diseases in domestic ruminants (cattle, sheep, and goats) [32]. The prevalence of *Fasciola hepatica* in Tunisia affects approximately 14.3% of cattle [17] and between 17.1 and 23.8% of goats in Morocco [20]. In Algeria, epidemiological surveys carried out at slaughterhouses revealed

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percentages of 52.4% in cattle at El Taref [9], 27% at Jijel and 9.1% at Constantine [23]. Serological surveys carried out on a few farms showed a frequency of 6.3% in Constantine and 43.7% in Bouinan [3]. The cosmopolitan distribution of this parasitosis affects both northwest and northeast Algeria.

The ecological conditions (climatic and ecopedological conditions) of the fluvial zones favour the proliferation of mollusks, and the presence of the intermediate host (*Galba truncatula*) in trampling areas is a link in the overall infestation pattern.

Given its elective location, liver is the seat of many syntheses that expose it to many major metabolic disorders, causing economic losses such as weight loss and decreased milk production.

Frequently, the chronic and silent aspect of fasciolosis is only discovered in the slaughterhouse [16].

In this context, the objective of our study was to evaluate the parasitic status of livestock in north eastern Algeria. Although the coprological diagnosis is rapid and easy, it is often conditioned by the presence and/or absence of *Fasciola hepatica* eggs. Their intermittent excretion undeniably suggests a confirmation by an immuno-enzymatic test. For this, we combined liver markers to evaluate the liver damage. Thus, a histopathological study of the liver was performed to visualise the chronological pattern of the infection.

## Materials and Methods

### Study Area

The study was performed from July to November 2015 at privately owned farms located in northeastern Algeria (Fig. 1). The prevailing climate at the time of the study as subhumid, with 70% of humidity, influenced by a high average rainfall in winter (168.40 mm) and a high temperature in summer (26 °C) <https://www.onm.org.dz> (National Meteorological Office-Avenue Khemisti, Dar El Beida, BP 153, Alger, Algeria). The experimental farms are located on uneven ground that may contain *Galba* because of their proximity to water points (lakes).

### Experimental Groups

The survey was conducted randomly on a total of 143 cattle, 15 males, and 128 females, from different breeds: Holstein ( $n=45$ ), Montbeliard ( $n=74$ ), crossed ( $n=22$ ), indigenous ( $n=1$ ), and Norman ( $n=1$ ). The animals included in the experiment were 1–10 years.

## Study Design and Sampling

We simultaneously collected 143 faeces and blood; the faeces were taken from the rectum of each animal, placed in plastic bags and labelled. The presence of *F. hepatica* eggs in faecal samples was evaluated by a modified sedimentation method [18]; briefly, 250 g of faeces were mixed in 1 L of tap water and filtered through a tea sieve. The glass content was sedimented for 30 min, stained with methylene blue and then microscopically observed at a magnification of  $\times 40$ .

Blood samples were collected from the jugular vein, centrifuged at 3000 rpm for 10 min, and sera was recovered and stored at  $-20$  °C until used for biochemical analysis and antibody detection.

Determination of the transaminases (Alat: alanine aminotransferase, Asat: aspartate Aminotransferase), alkaline phosphatase (Pal), gamma glutamyltransferase ( $\gamma$ GT), albumin, and total protein was performed on commercially available kits (BIOLABO SAS, Matzy, France). The working solutions were prepared in accordance with the supplier's recommendations.

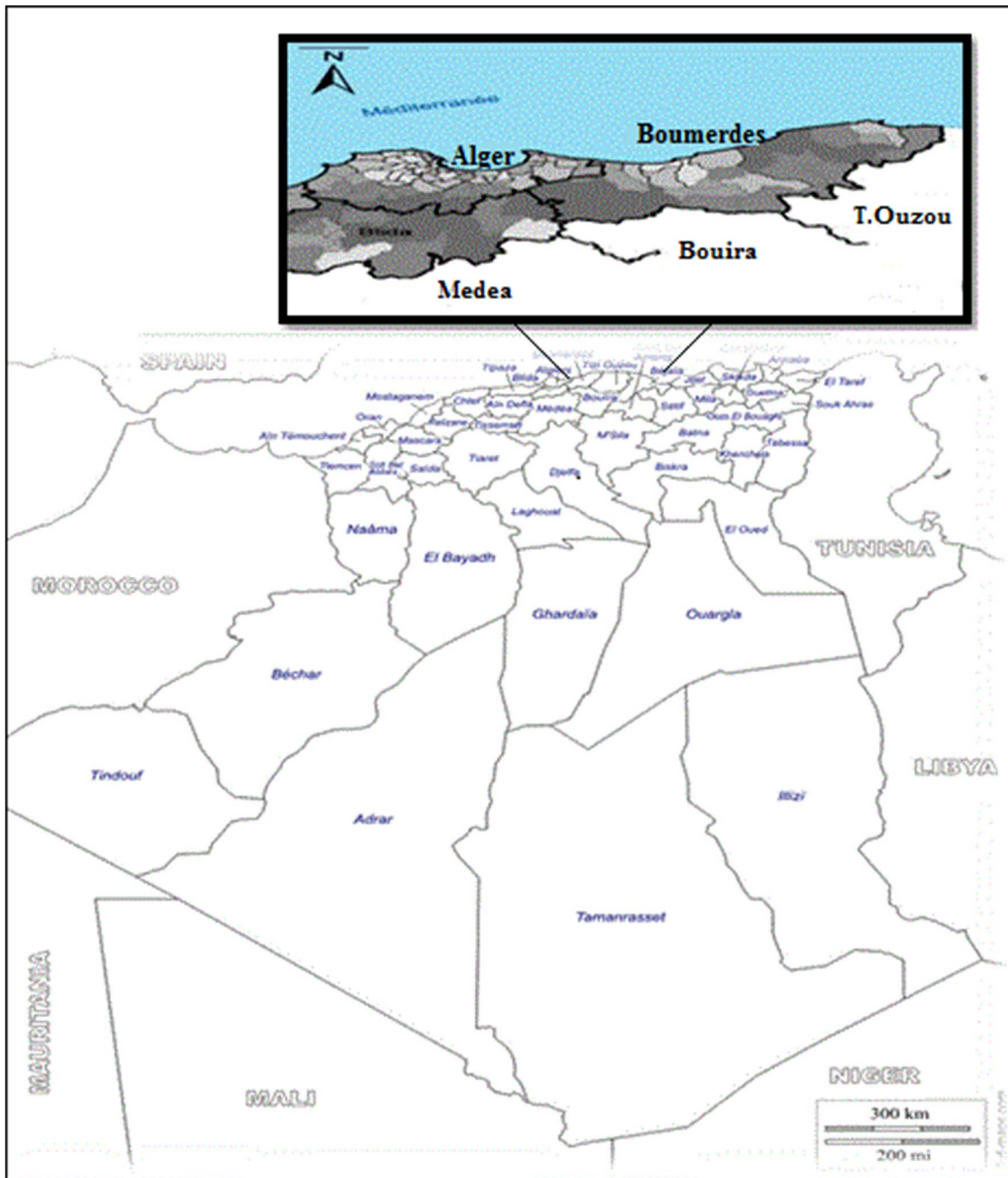
## Immunological Diagnosis of Fasciolosis in the Serum via the ELISA Methods

Serum IgG antibodies were assayed using specific, commercially available *F. hepatica* antigens (IDEXX Fasciolosis Verification, Montpellier, France). Microplates were coated in alternance with control antigen ( $-Ag$ ) and f2 antigen ( $+Ag$ ) in both odd and even columns, respectively; 10  $\mu$ l of sera in duplicate were added to each well without dilution and incubated for 1 h at 37 °C. After washing, 100  $\mu$ l of peroxidase-conjugated anti-bovine IgG antibody were incorporated in each well and incubated 30 min at 37 °C to bind to the immune-complex antigen–antibody. After another washing, 100  $\mu$ l of the enzyme–substrate were added to the conjugate (18–26 min protected from light), resulting in the formation of a blue compound which becomes yellow after blocking (100  $\mu$ l). Optical density (OD) was read at 450 nm, using a plate reader (Bio-Rad, USA). The diagnosis was established by comparing the net optical density of the sample with the mean net optical density of the positive control, according to the following equation:

### Calculation of Positive Control

$$CP\bar{X} = (CP1_{+Ag} A(450) + CP2_{+Ag} A(450))/2$$

$$NE\bar{X}_{CP} = [(CP1_{+Ag} A(450) - CP1_{-Ag} A(450)) + (CP2_{+Ag} A(450) - CP2_{-Ag} A(450))]/2$$



**Fig. 1** Representative map of the study area

$$NE_{CN} = CN_{+Ag} A (450) - CN_{-Ag} A (450),$$

where  $CP\bar{X}$  is the average optical density value of the positive control,  $NEX_{CP}$  is the average net optical value of the positive control, and  $NE_{CN}$  = average net optical value of the negative control.

**Calculation of the Sample**

$$NE = E_{+Ag} A (450) - E_{-Ag} A (450) \dots \dots E/P \% = 100 \times (NE/NEX_{CP}),$$

where NE is the net optical value.

## Histopathology Assessment

One bovine liver with fasciolosis, obtained from the slaughterhouse of Thenia, was used. One 18-month-old Montbeliard cattle (male) was obtained from one of the farms studied and sacrificed in the presence of the veterinary inspector of the department. The presence of *F. hepatica* was confirmed macroscopically. A fragment of hepatic lobe was seized, fixed in 10% of neutral buffered formalin, and embedded in paraffin. Sections (5 µm) were stained with Goldner's modified Masson Trichrome to determine the damaged liver areas. To obtain more details, we analysed the histological hepatic fragment via FEI Quanta 250 scanning electron microscopy (SEM/EDX). The samples were not pre-treated,

and the images were taken at 10.00 kV in a low-vacuum (10–130 Pa) secondary electron (LFD) detector at a magnification of ×41 to 600.

## Statistical Analysis

The data were initially organised on an excel spreadsheet and then imported into the XLSTAT statistical SOFTWARE (Version 2017). The prevalence to *F. hepatica* was calculated as a percentage value, and analysis of variance (ANOVA) was used to determine the statistical relationship between infection rates and three epidemiological risk factors (age, sex, and breed).

**Table 1** Summary table showing the distribution of age, sex and breed in the 13 farms studied

Farms	Sex	Breed	Age (months)	Eggs
A (n = 13)	Males (n = 0)/females (n = 13)	Montbeliard: n = 7 Holstein: n = 5 Crossed: n = 1	12–120	Absence
B (n = 13)	Males (n = 0)/females (n = 13)	Montbeliard: n = 12 Holstein: n = 0 Crossed = 1	24–48	Absence
C (n = 22)	Males (n = 0)/females (n = 22)	Montbeliard: n = 12 Holstein: n = 10 Crossed = 0	18–120	Absence
D (n = 19)	Males (n = 11)/females (n = 8)	Montbeliard: n = 10 Holstein: n = 2 Crossed: n = 5 Indigenous: n = 1 Norman: n = 1	12–60	Presence
E (n = 10)	Males (n = 1)/females (n = 9)	Montbeliard: n = 9 Holstein: n = 1 Crossed: n = 0	14–120	Absence
F (n = 10)	Males (n = 0)/females (n = 10)	Montbeliard: n = 9 Holstein: n = 1 Crossed: n = 0	12–96	Absence
G (n = 4)	Males (n = 1)/females (n = 3)	Montbeliard: n = 3 Holstein: n = 1 Crossed: n = 0	12–72	Presence
H (n = 3)	Males (n = 1)/Females (n = 3)	Montbeliard: n = 0 Holstein: n = 1 Crossed: n = 2	12–72	Absence
I (n = 6)	Males (n = 0)/females (n = 6)	Montbeliard: n = 3 Holstein: n = 1 Crossed: n = 2	12–72	Absence
J (n = 3)	Males (n = 0)/females (n = 3)	Montbeliard: n = 0 Holstein: n = 1 Crossed: n = 2	72–120	Presence
K (n = 7)	Males (n = 0)/females (n = 7)	Montbeliard: n = 2 Holstein: n = 2 Crossed: n = 3	36–180	Presence
L (n = 17)	Males (n = 0)/females (n = 17)	Montbeliard: n = 4 Holstein: n = 7 Crossed: n = 6	12–96	Absence
M (n = 16)	Males (n = 1)/females (n = 15)	Montbeliard: n = 3 Holstein: n = 13 Crossed: n = 0	18–84	Absence

**Table 2** Prevalence of fasciolosis in north eastern farms (A–M) of Algeria according to age, sex, breed and *F. hepatica* eggs based on the serological results of anti-f2 antibodies

Samples n=143	Prevalence of infestation (%)					p value			
	No	Low	Average	Strong	Total	Age	Sex	Breed	<i>Fasciola hepatica</i> eggs
A (n=13)	100	0	0	0	22.37%	p=0.018	NS	NS	p<0.0001
B (n=13)	100	0	0	0		NS	NS	NS	
C (n=22)	40.91	18.18	27.27	13.64		NS	NS	NS	
D (n=19)	63.16	0	10.53	26.31		NS	NS	NS	p=0.0003
E (n=10)	90	10	0	0		NS	p<0.0001	p<0.0001	
F (n=10)	100	0	0	0		NS	NS	NS	
G (n=4)	25	25	50	0		p=0.002	NS	NS	p=0.056
H (n=3)	100	0	0	0		NS	NS	NS	
I (n=6)	66.67	0	16.67	16.67		p=0.001	NS	NS	
J (n=3)	33.33	0	66.67	0		NS	NS	NS	NS
K (n=7)	71.43	14.28	14.28	0		NS	NS	NS	NS
L (n=17)	94.12	0	0	5.88		NS	NS	NS	
M (n=16)	93.75	6.25	0	0		NS	NS	NS	

NS non significant, p value probability

Multiple comparison tests were performed to compare the immunological response to infection (serological presence of antibody anti-f2 of *F. hepatica*) and hepatic enzymes, including both protein and albumin rates, between farms (A to M).

The relationship between variables was considered statistically significant at a p value of less than 0.05, with a 95% confidence level.

## Results

### Relationship Between Hepatic *Fasciola hepatica* Infection and Changes in Serum Parameters

To investigate the serological prevalence to *F. hepatica* infection, 143 cattle from 13 farms were tested. The details of the distribution of the population in terms of age, sex, and breed are summarised in Table 1. The ELISA test results showed a heterogeneous distribution of the anti-f2 antibodies in response to the infection. As depicted in Table 2, the overall infestation rate was about 22.37%, which corresponds to a ratio of 0.69; i.e., 9 farms out to 13 were affected. We observed three levels of infestation, as described in Table 2. The most affected farms reached levels exceeding 50%, as recorded for farms C (59.09%), G (70%), and J (66.67%). Farms A, B, F, and H were free of this zoonosis (100%).

Serological confirmation of *F. hepatica* infection has broadened the study by integrating multiple combinations referring to age, sex, and breed. Based on the statistical analysis, there was a significant difference related to age (p=0.018) and the presence of *F. hepatica* eggs (p<0.0001). Regarding sex and breed, no significant difference was recorded for the 143 samples studied.

The factors mentioned above suggest their involvement in the appearance of the infection observed in farms.

Table 3 shows the results of the multiple comparison test, which was performed to obtain the in vivo state by setting the dependent (anti-f2 antibodies) and explanatory (age) variables by integrating faecal eggs, breed and sex into the equation.

In each column selected, significant effects were noticed at different degrees because of the importance of integrated variables. The first column indicates that the presence of faecal eggs reflects the active infectious status of cattle (p<0.0001), while age (p=0.011) and sex (p=0.040) will increase chance of acquiring the infection.

This was identical for all the categories studied except for column 5, where no explanatory variable (age) was selected.

The clinical value of the determination of serum parameters relates to the exploration of liver function. In fact, the most commonly observed variations affect total proteins including anti-f2 antibodies and albumin. Because some



**Table 3** Analysis of differences between modalities (faecal eggs, breeds and sex) by fixing the dependent and explanatory variables: anti-f2 antibodies and age, respectively

		Fixed dependent variable (anti-f2 antibodies)				
Explanatory variable						
Age	$p=0.011$	$p=0.063$	$p=0.002$	$p=0.011$	–	
Qualitative variables						
Faecal eggs	$p<0.0001$	$p<0.0001$	$p<0.0001$	–		$p<0.0001$
Breed	NS	NS	–	NS		NS
Sex	$p=0.040$	–	$p=0.051$	$p=0.017$		NS

enzymes are interesting from a lesional point of view, we confined the investigations to analysis of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyltransferase ( $\gamma$ GT), and alkaline phosphatase (PAL).

Table 4 shows the expression of the serological parameters divided into two categories: proteins (anti-f2 antibodies, total proteins, and albumin) and hepatic enzymes according to age. With the exception of IgG, which was statistically significant ( $p=0.018$ ) for all cattle studied, proteins and albumin were not significant. Table 4 indicates that the mean serum value of the anti-f2 antibodies increases equivalently in the farms C, D, G, and J, affected by fasciolosis. The maximum percentages were observed on farms G ( $79.75 \pm 37.64\%$ ,  $p=0.002$ ) and J ( $79.76 \pm 40.58\%$ ), while minimum values were recorded on farms A ( $3.18 \pm 1.49\%$ ), B ( $3.86 \pm 1.41\%$ ), F ( $1.14 \pm 0.25\%$ ), and M ( $3.06 \pm 2.03\%$ ). Table 4 also shows the slight fluctuations in the distribution of total proteins between different farms. The impact of fasciolosis on the profile of total proteins is similar to that on albumin, albeit with some exceptions. The average ranges were between  $107.56 \pm 5.54$  and  $69.45 \pm 5.50$  g/l for farms A and G. Albumin levels were highest and lowest for farms F and G, respectively, with  $806.75 \pm 75.04$  and  $363.26 \pm 42.93$   $\mu$ mol/l. Unlike albumin, which showed no significant difference within farms and for the entire herd, proteins were significantly different within farms B ( $106.71 \pm 3.79$  g/l,  $p=0.049$ ), H ( $76.00 \pm 7.31$  g/l,  $p=0.005$ ), and I ( $97.77 \pm 4.97$  g/l,  $p=0.004$ ).

The responses of liver enzymes to the parasitic infection are summarised in Table 4; average values of 143 cattle were below the standard. However, for all liver enzymes, only PAL was highly significant ( $p<0.0001$ ) for age. Subjected to this parameter, a maximum ( $123.89 \pm 56.41$  UI/l) and minimum levels ( $42.82 \pm 3.76$  UI/l) were observed, respectively, for farms H and K. Fasciolosis also causes the elevation of AST, as seen in farms G ( $73.77 \pm 28.62$  UI/l) and K ( $67.84 \pm 8.76$  UI/l), suggesting liver injury in terms of individual values. The results also showed that farm D was statistically significant for PAL ( $101.85 \pm 12.80$  UI/l,  $p=0.015$ ),  $\gamma$ GT ( $13.25 \pm 1.58$  UI/l,  $p=0.041$ ) and ALT ( $6.63 \pm 0.62$  UI/l,  $p=0.009$ ).

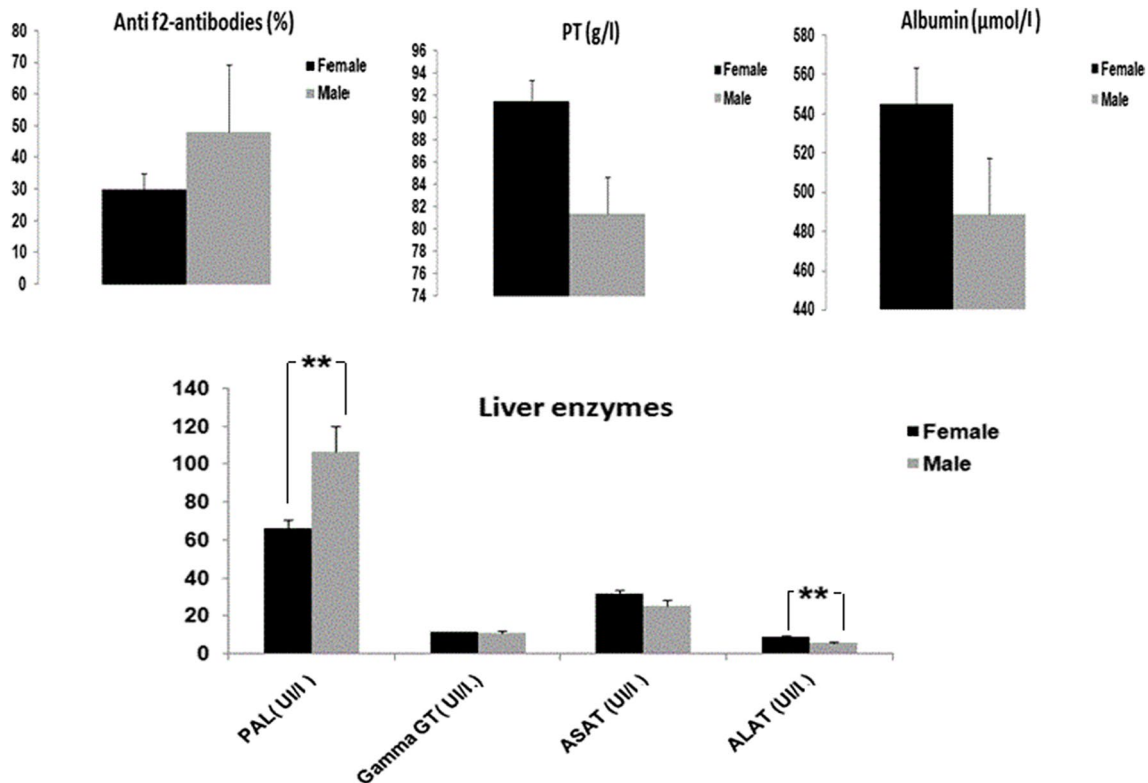
Another aspect explored in our study was the effect of parasitosis on the liver enzymes released. This approach allowed us to note that  $\gamma$ GT ( $r=0.401$ ,  $p<0.0001$ ) and AST ( $r=0.186$ ,  $p=0.027$ ) responded significantly to the infection. The different farms showed differences in terms of parameters. For example, for farm A, we observed a highly significant liver production of albumin ( $p=0.004$ ) in response to circulating IgG levels during the infection–secretion cycle. This tendency was expressed differently in farms B ( $p=0.009$ ) and D ( $p=0.001$ ), where  $\gamma$ GT did not reach extremely high, but still significant levels. Unlike farm I (total proteins:  $p=0.013$ , AST:  $p=0.038$ , ALT:  $p=0.046$ ), which perfectly expressed the infectious power of distomatosis, the remaining farms (C, E, F, G, H, J, K, L, and M) seemed to be unscathed ( $p>0.05$ ).

Regarding sex, no significant differences were recorded for anti-f2 antibodies, proteins, albumin,  $\gamma$ GT, and AST, except for both PAL ( $p=0.005$ ) and ALT ( $p=0.005$ ). In our study, males were more infected (27%) than females (22%), as shown in Fig. 2. Although the numerical superiority of females is established (128 females vs. 15 males), only 10% of the males of the farm E showed a significant difference ( $p<0.0001$ ) in terms of anti-f2 antibodies to *Fasciola hepatica* (Fig. 3). A relatively close infestation rate between males (15.79%) and females (26.31%) was observed for farm D. In addition, farms C, D, G, I, J, K, L, and M showed infestation levels of females ranging from 6 to 75%.

The post-analysis results of the 143 samples showed that five distinct breeds [Montbeliard (M), Holstein (H), Crossed (C), Norman (N), and Indigenous (I)] forming herds, with some marked racial disparity among the 13 farms. In view of the results shown in Fig. 4, the Holstein breed appears to be the most affected (31.11%), followed by the Montbeliard (20.27%) and the crossed breed (13.64%). With the exception of ALT ( $p=0.027$ ), no significant difference was observed for breed. As illustrated above (Fig. 5), farm E showed an infestation rate of 10%, with a highly significant difference ( $p<0.0001$ ) for the Holstein breed. In addition, we observed the following details: C (M 27.27% vs. H 31.82%); D (M 26.31% vs. H 5.26% vs. C 5.26%); G (M 50% vs. H 25%); I (M 16.67% vs. H 16.67%); J (H 33.33% vs. C 33.33%); K (M 14.28% vs. C 14.28%); L (H 5.88%);

**Table 4** Serological parameters (X ± SEM) after *F. hepatica* infection according to the age in northeastern farms of Algeria

Serological parameters												
Farms	Anti-F2 antibodies (%)	Total proteins g/l	Albumin (μmol/l)	Pal (UI/l)	YGT (UI/l)	AST(UI/l)	ALT (UI/l)					
A	3.18 ± 1.49 NS p=0.018	107.56 ± 5.54 NS	423.30 ± 21.84 NS	84.52 ± 18.67 p=0.050	70.20 ± 4.46 p < 0.0001	11.34 ± 0.36 NS	23.95 ± 2.15 NS	31.23 ± 1.58 NS	9.59 ± 1.25 NS	8.65 ± 0.37 NS		
B	3.86 ± 1.41 NS	106.71 ± 3.79 p=0.049	408.65 ± 18.61 NS	55.54 ± 7.09 p=0.044	9.58 ± 0.49 NS	25.83 ± 2.01 NS	8.34 ± 0.77 NS					
C	72.72 ± 15.48 NS	92.78 ± 3.02 NS	554.89 ± 20.69 NS	65.07 ± 15.30 NS	11.65 ± 0.66 NS	34.85 ± 3.02 NS	10.21 ± 1.03 NS					
D	73.87 ± 23.11 NS	92.73 ± 5.20 NS	476.50 ± 17.71 NS	101.85 ± 12.80 p=0.015	13.25 ± 1.58 p=0.041	23.20 ± 2.37 NS	6.63 ± 0.62 p=0.009					
E	7.43 ± 5.32 NS	74.83 ± 3.00 NS	367.43 ± 26.34 NS	70.03 ± 10.93 p=0.067	10.33 ± 0.65 NS	18.52 ± 1.69 NS	5.60 ± 0.93 NS					
F	1.14 ± 0.25 NS	101.7 ± 6.08 NS	806.75 ± 75.04 NS	45.61 ± 6.12 NS	9.00 ± 0.76 NS	26.87 ± 2.09 NS	7.65 ± 0.87 NS					
G	79.75 ± 37.64 p=0.002	69.45 ± 5.50 NS	363.26 ± 42.93 NS	51.23 ± 3.83 NS	12.51 ± 2.20 NS	73.77 ± 28.62 NS	7.65 ± 0.87 NS					
H	5.56 ± 3.44 NS	76.00 ± 7.31 p=0.005	395.57 ± 36.55 NS	123.89 ± 56.41 NS	9.40 ± 1.20 NS	28.52 ± 2.54 NS	4.42 ± 0.41 NS					
I	57.81 ± 32.77 p=0.001	97.77 ± 4.97 p=0.004	366.03 ± 23.87 NS	96.74 ± 33.25 NS	9.79 ± 0.78 NS	30.15 ± 5.85 p=0.055	11.35 ± 0.89 NS					
J	79.76 ± 40.58 NS	81.4 ± 3.47 NS	369.75 ± 68.62 NS	71.39 ± 11.24 NS	10.32 ± 0.82 NS	41.32 ± 9.80 NS	10.07 ± 3.31 NS					
K	28.43 ± 12.83 NS	80.16 ± 11.47 NS	389.77 ± 24.82 NS	42.82 ± 3.76 NS	16.24 ± 2.64 NS	67.84 ± 8.76 NS	14.91 ± 2.21 NS					
L	12.66 ± 9.58 NS	84.27 ± 4.29 NS	695.89 ± 29.21 NS	54.80 ± 8.40 NS	13.97 ± 0.99 NS	38.37 ± 2.69 NS	8.71 ± 1.18 NS					
M	3.06 ± 2.03 NS	78.37 ± 2.99 NS	796.18 ± 55.71 NS	68.26 ± 12.28 p=0.001	9.50 ± 0.49 NS	22.53 ± 2.89 NS	7.03 ± 0.83 NS					



**Fig. 2** Evaluation of levels of *Fasciola hepatica* anti-f2 antibodies, total proteins, albumins and liver enzymes in response to *F. hepatica* infection according to sex. Results are expressed as  $X \pm SEM$

and M (H 6.25%). The other farms (A, B, F, and H) had healthy animals.

### Histopathological Alterations and Changes in Liver Tissue

Microscopic observation (Fig. 6) showed a section of healthy liver (a, b, c, and d) with hepatocyte lobules arranged radially around the central vein (a), forming Remak bays separated by sinusoidal channels and delimited by the Glisson's capsule (b). At the contact angles of the hepatic lobules are the portal spaces (c and d): the triad (portal vein, bile duct, and hepatic artery).

Figure 7 shows the architectural changes of a parasitised liver, caused by the presence of *Fasciola hepatica*. The pathogenic process shows the formation of fibrous trabeculae between the hepatic lobules, defining annular and biliary fibrosis evolving towards a sclerosis (a). The absence of the centrilobular vein would explain the presence of regeneration lobules following a partial destruction of the hepatic parenchyma (b). The periportal fibrosis (c) progresses to the formation of porto-portal bridges separating the hepatic parenchyma in nodules (dissection nodules). Hepatocytes trapped in the connective tissue septa form cholangioles (d

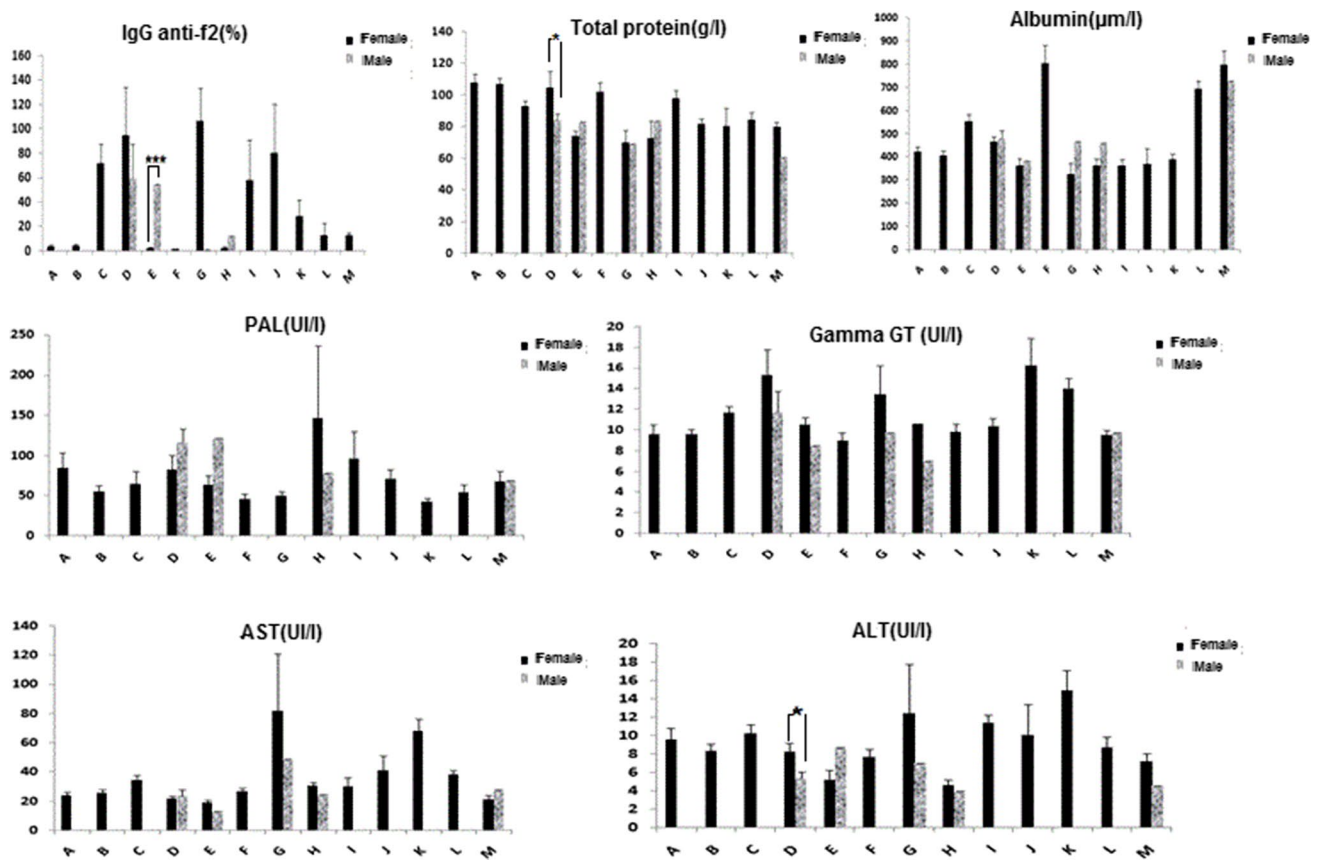
and e). The histological aspect of Fig. 7f refers to a diffuse portal leukocyte infiltrate indicative of a response to parasitism. Trapped in the bile ducts (Fig. 7g, h and i), liver fluke appears as debris. The scar tissue (fibrosis) observed around the liver lobules suggests chronic hepatitis.

Figure 8 shows bile duct glandular epithelium proliferation, forming a new bile ductule surrounded by thick connective tissue (a). Indeed, portal fibrosis showed liver fluke in the lumen of the intra-hepatic bile duct, destroying the bile duct (b) with heavy infiltration of inflammatory cells (c, d, and e) in the fibrous connective tissue between newly formed bile ductules.

As depicted in Fig. 8f, g, h, and i, a yellowish longitudinal acellular form was observed at different magnifications, infiltrating the hepatic parenchyma and causing its disorganisation. In the centre, cellular debris surrounding an amorphous material was observed.

Macroscopic lesions observed in Fig. 9a, b, c, and d show a circumferential thickening of the wall of the bile ducts with narrowing of light, suggesting an inflammatory cholangiopathy. This ductular reaction is thought to be secondary to the presence of the liver fluke. The scanning electron microscopy (SEM) image (a1) of the biliary duct showed a dense structure containing a limestone formation, confirmed by the





**Fig. 3** Evaluation of levels of *Fasciola hepatica* anti-f2 antibodies, total proteins, albumins and liver enzymes in response to *F. hepatica* infection according to the sex of all animals of the 13 selected farms

(A–M). Results are expressed as  $X \pm \text{SEM}$ . PAL alkaline phosphatase, Gamma GT gamma glutamyl transpeptidase, AST aspartate aminotransferase, ALT alanine aminotransferase

pictures a2, a3, a4, and a5. In fact, the SEM mode allowed collecting fine structural and topological data, together with insights into the interior of the sample. In addition to the above-mentioned pathological calcifications (b1), porous formations (b2, b3, b4) appeared at the level of the bile ducts, suggesting a remodelling of the latter.

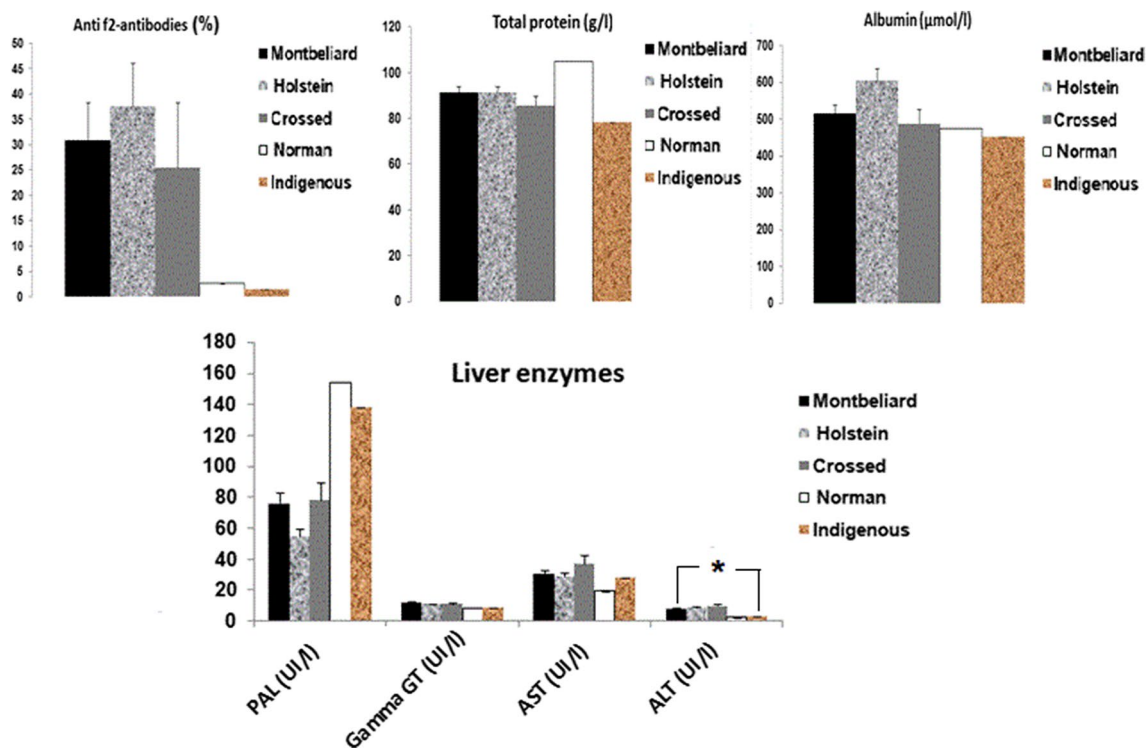
## Discussion

The evaluation of fasciolosis in cattle and its geographical distribution was the subject of our study. The northeastern areas of Algeria have a subhumid climate and border water reservoirs with the presence of snails. We focused on the upstream detection of zoonotic infections, which are often discovered in the slaughterhouse.

There is a great variability in the prevalence of natural *F. hepatica* infections in domestic ruminants between slaughterhouses and farms. Unlike the slaughterhouse model, farms show a heterogeneous distribution of infection, as reported in southwest Tunisia with 14.3% [17]. In the same way, the seroprevalence examined via a number of blood samples in

cattle revealed averages ranging from 6.3 to 35.5% in Algeria [23]. Similarly, the plain of Mitidja, with its different localities, showed an infestation rate close to 18.8% [3]. Morocco is also affected by this parasitosis, which is reflected by relatively high levels (49.1%). However, this prevalence has been determined according to the seasons [24]. Epidemiology of bovine fasciolosis in the Nile Delta region of Egypt does not exceed 9.77% [14]. All these data place fasciolosis around the Mediterranean basin, although it is not specific to underdeveloped countries, since it occurs in Romania (63%) [27], France (75%) [29], Equatorial Guinea (44.1%) [7], Turkey (65.2%) [35], and Black Africa (21.39%) [16].

For some countries cited above, this cosmopolitan zoonosis is closer to the values obtained in our study, because the serological investigations of *F. hepatica* infection were around 22.37%, according to their incidences on the different farms: C (59.09%), D (36.84%), E (10%), G (70%), I (33.34%), J (66.67%), K (28.56%), L (5.88%), and M (6.25%). The higher prevalence rate of fasciolosis in the present study might be due to the variation in agro-ecological conditions favouring snail occurrence.



**Fig. 4** Evaluation of levels of *Fasciola hepatica* anti-f2 antibodies, total proteins, albumins and liver enzymes in response to *F. hepatica* infection according to breed

Because hepatobiliary distomatosis is highly unobtrusive (a chronic form in cattle), many cases of fasciolosis have been reported in several slaughterhouses during veterinary inspections. Slaughterhouse discoveries provide information on the frequency of this lesion and its extent. In Haiti, for example, 10.67% of cattle killed confirmed this parasitosis [8]. In northeastern Nigeria, the overall percentage of *Fasciola gigantica* antibodies detected was around 72%, distributed seasonally [11]. On the basis of post-mortem examinations, in the industrial abattoir located in Gondar (northwestern Ethiopia), 79.83% of the infection were due to *F. hepatica*, with a near-normal distribution [43.37% (2005), 70.68% (2006), 53.44% (2009), and 52.35% (2010)] between 2005 and 2010 [25]. The picture that we describe here is far from being exhaustive, since Algeria has extended this list with natural infections to *Fasciola* in cattle and sheep from slaughterhouses of Constantine (9.1%) and Jijel (27.0%) [3]. Countries such as USA are not unscathed to this parasitosis, and fasciolosis is quite present, with nine cases reported in humans [4].

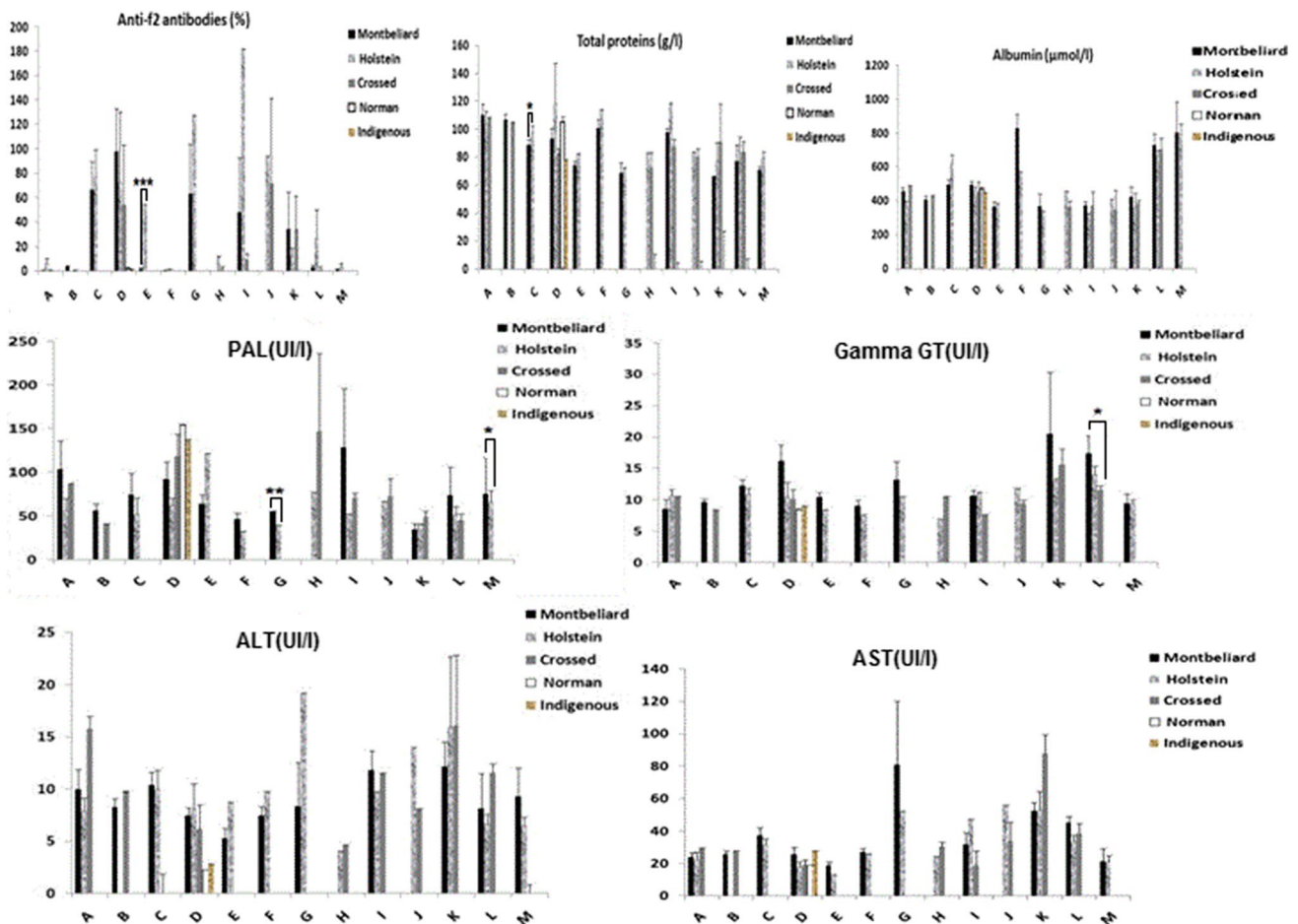
The abattoir and areas with a prevalence of fasciolosis fluctuate considerably between strangely high and relatively low values. This heterogeneity can be associated with numerous factors, such as animal breeding sites, favourable ecological biotopes, climate, age, soil ecopedology, or the lack of livestock management systems

[9]. Beyond classic and known data on infestations, other approaches have been considered, such as the study of snail populations and snail age to explain this variability [12].

Unlike previous studies, we can confirm that our herd has not benefited from any anthelmintic treatment, which is an excellent indicator of the infestation level.

Although the coprological examination shows its own limitations, it remains consistent with the serological data on the infection (presence of anti-f2 antibodies against *F. hepatica*) in our study. However, the opposite is not necessarily right.

To explain the intrinsic variability to the infection (anti-f2 antibodies), we independently integrated age, sex, and breed as explanatory variables. In a previous study, age was an important factor for the prevalence of fasciolosis in ruminants [15]. We observed that age greatly influenced the prevalence of the infection, which was expected. Similar results have been described by [35], who report that older animals  $\geq 6$  years were more infected than young animals  $\leq 2$  years, which was the case at least for sheep because of the relaxation of their immunity [2]. Our findings are in agreement with those of [24], who consider older sheep as the main reservoir of this parasitosis. In view of our results, we find that the degree of infestation with *F. hepatica* on the basis of breed showed no statistically significant difference.



**Fig. 5** Evaluation of levels of *Fasciola hepatica* anti-f2 antibodies, total proteins, albumins and liver enzymes in response to *F. hepatica* infection according to the breed of the animals of the 13 selected farms (A–M)

The infection rate in the Holstein breed (31.11%) was higher than that in the Montbelliard breed (20.27%) and the crossed breed (13.64%), which could be due to the genetic potential of the Holstein breed because of its significant milk production, making it more sensitive. Our results disagree with a study performed in Ethiopia, which favours the local breed in terms of infections [34]. In regard to the effect of sex and despite the fact that females were numerically superior (15 male for 128 females), the infestation rates of males were higher than those of females (27% vs. 22%). Most likely, this can be explained by the fact that most female cattle are kept for lactation. However, there was no statistically significant difference in infection rates between males and female, as described by [34]. We conclude the independent of the risk factors (age, sex, and breed) in the expression of *F. hepatica* parasitism.

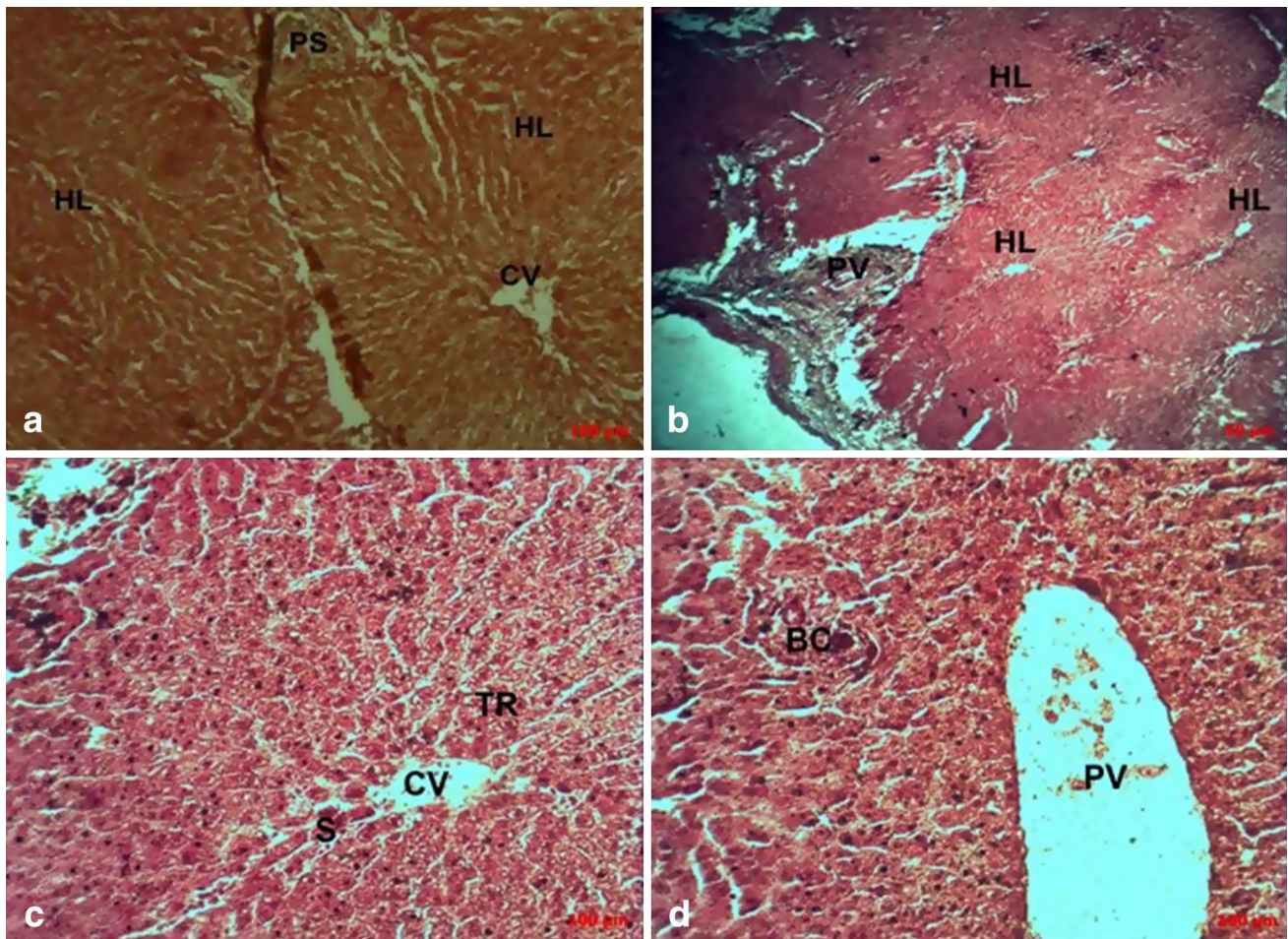
The identification of potential risk factors for liver fluke infection has been complicated by setting independent variables one by one. However, based on multivariate modelling calculations, we were able to demonstrate that age,

sex, breed, and coprology provided a wealth of information about their relevance. The model in column no. 1 (Table 3) represents the best state encountered in vivo, most closely reflecting the field conditions.

The macroscopic observation, the histopathology, and the serological detection of anti-f2 antibodies as well as liver enzymes are concordant, which allows us to attribute the majority of observed liver lesions to distomic infestations that mark their chronicity.

The macroscopic liver lesions caused by fasciolosis in infected cattle showed an increase in liver volume, with a lighter colour. White-haired longitudinal trabeculae were present on the surface of the parenchyma, and there was a screeching during the post-mortem examination of the organ, possibly caused by calcareous structures. In addition, many canalicular lesions referring to the bile ducts showed thickened walls containing liver flukes of different sizes. Our visual observations were similar to the pathological investigations of liver fluke infections of slaughtered black Bengal goats in Bangladesh [31].





**Fig. 6** Liver biopsy showing the juxtaposition of hepatic lobules (a magnification 10× and c magnification 20×) and portal triad (b magnification 4× and d magnification 20×) in a healthy bovine liver

Microscopically, the histological lesions are characterised by diseased fibrosis around hepatic lobules, forming junctions between the portal spaces and leading to the deconstruction of the hepatic parenchyma. There is also compensatory hyperplasia of the bile ducts secondary to the infection with *Fasciola hepatica*, as described by [28]. The most prevalent alteration in chronic fibrosis was the proliferation of the bile duct glandular epithelium, forming new bile ductules surrounded by thick connective tissue. Similar results have been reported by [13] at the portal space and, more specifically, around the bile duct, an inflammatory reaction with eosinophils and neutrophils was observed, suggesting an immune response of the host during the migration of the young fluke, as also reported by [21] and [1]. Variable degrees of proliferation and calcification were also observed in the lumen of biliary ducts, revealing chronic infection. Our results are in agreement with two previous studies [5, 33].

Hepatic enzyme activities and biochemical variables in examined cattle serum show parasitic infestation related to

recovered at the slaughterhouse and used as a negative control, coloured with Masson's Trichrome

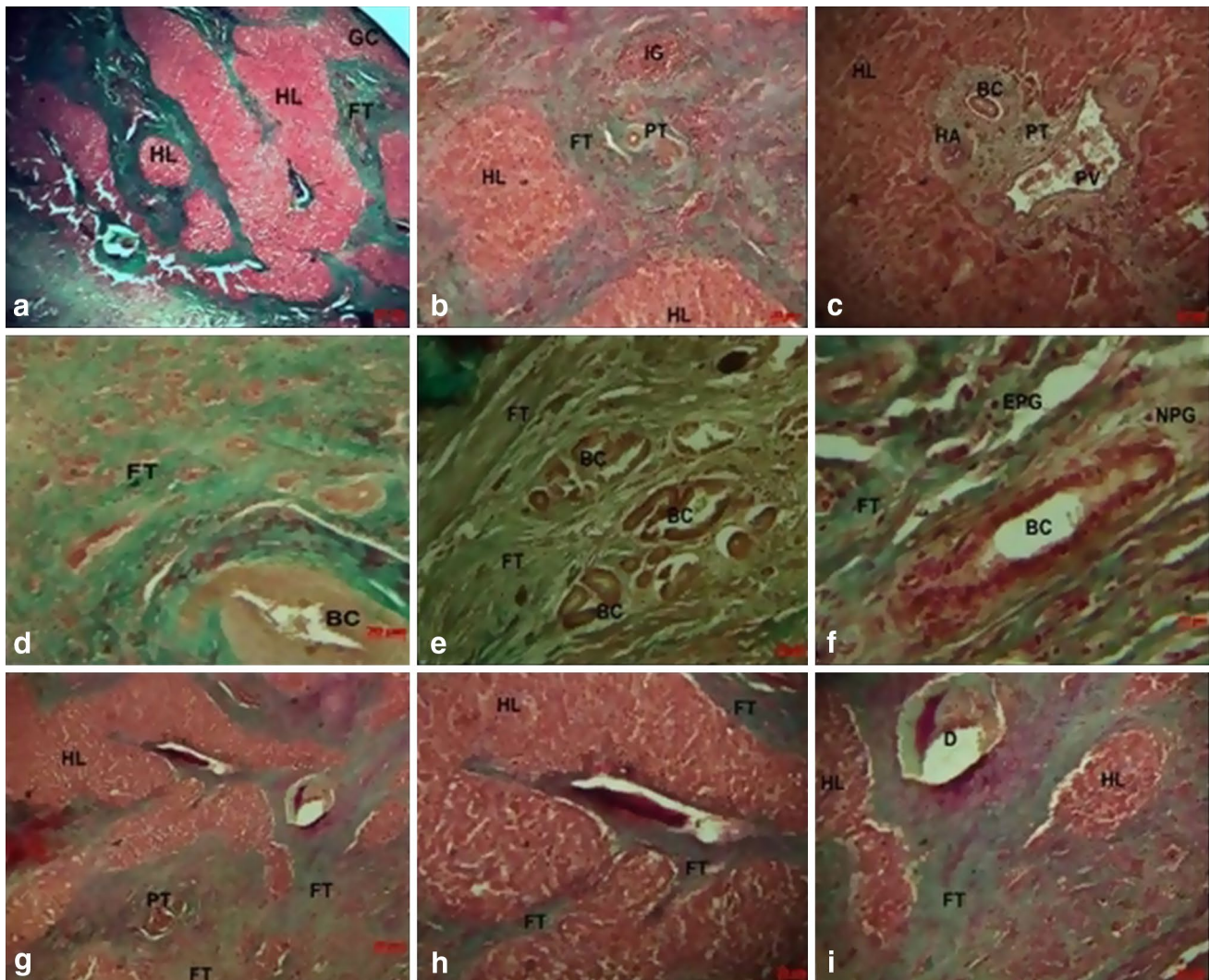
histopathology examination. Although AST and ALT are not specific for liver disease, they can be used in combination with other enzymes (PAL and  $\gamma$ GT) to monitor the course of various liver disorders.

The serological results reported in our study enabled us to evaluate the integrity of the liver parenchyma. Indeed, the relatively high levels of total proteins ( $90.68 \pm 1.67$  g/l) and albumin ( $539.02 \pm 17.01$   $\mu$ mol/l), except for some farms (F, L and M), can explain the inflammation [6], even though some means (albumin) recorded remain low with the example of the farms E, G, H, I, J, and K (see Table 4), indicating a discrete, reversible hepatic insufficiency.

In the chronic form, adult flukes cause cholangitis by attracting inflammatory cells, which is consistent with histopathology.

As previously described, hepatobiliary distomatosis did not completely destroy the hepatic parenchyma with regard to the few disseminated hepatic lobules, testifying to a brief regeneration of the latter, which was confirmed by the





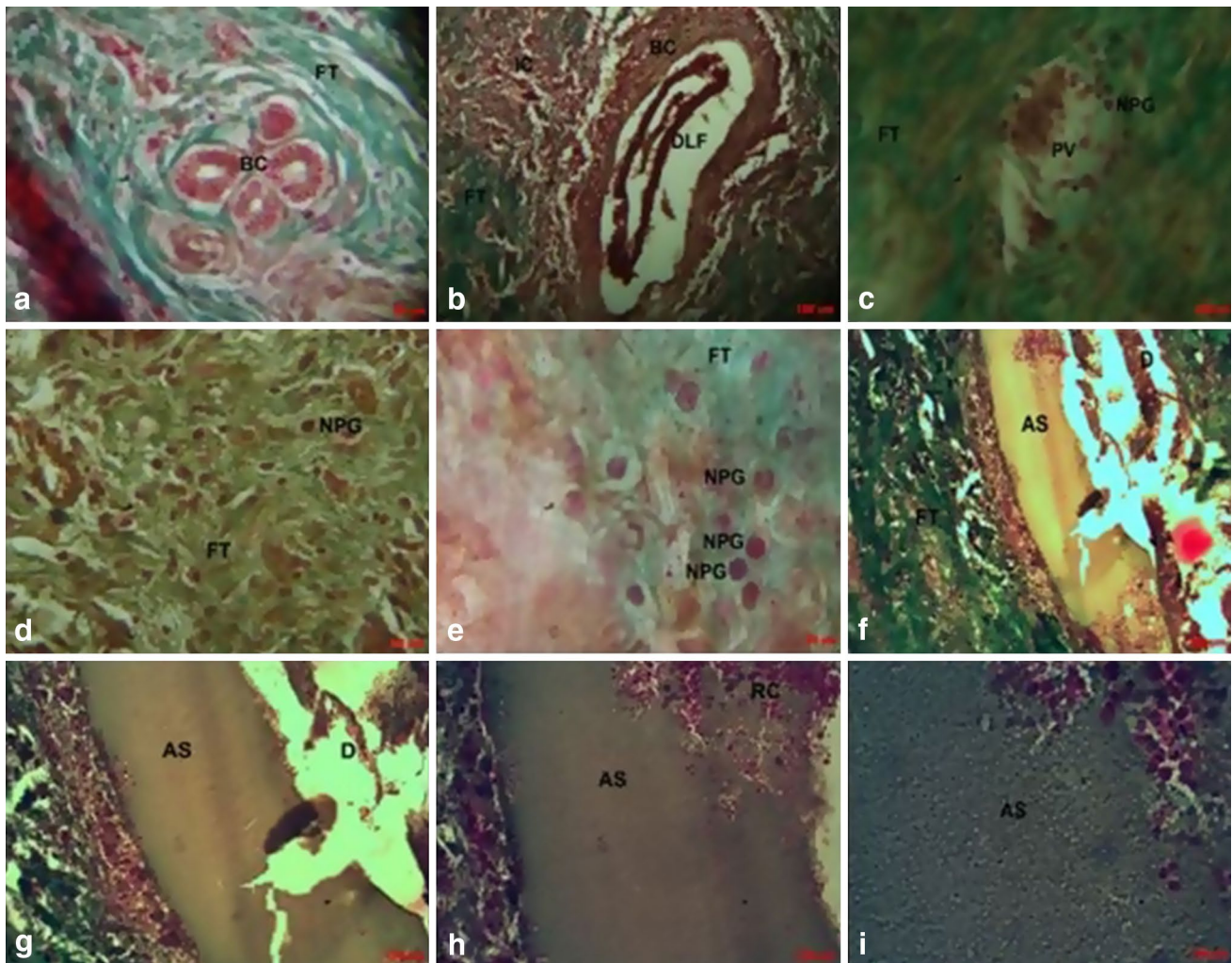
**Fig. 7** Liver biopsy showing a disruption of liver integrity (a 4×, b: 10×, c 4×, d 10×, e 10×, f 40×, g 4×, h 10×, i 10×), with a large periportal and lobular leukocyte

absence of the centrilobular vein. Our results contradict the work of [6, 13], which situates proteins below the reference levels, indicating hypoproteinemia secondary to liver disease with protein synthesis deficiency.

The activity of liver enzymes is determined according to the pathogenesis of the disease. Thus, to validate the chronic and/or the acute form, it seemed interesting to observe the variations in  $\gamma$ GT and PAL, which are canalicular enzymes. The hypothetical hepatic disturbances expected by the biochemical analysis place  $\gamma$ GT within the physiological limits (9.6–39 IU/l) in the same way as PAL (27–107 IU/l) [22], with certain individual exceptions in C3 (256.15 IU/l), D16 (179.85 IU/l) and E7 (121.53 IU/l), where we encountered very high values, suggesting biliary malfunction (cholestasis).

This difference is due to the great intra-species variability as described previously [26], and such isolated cases are

scattered throughout the flock. These enzymes, which are highly sensitive to *F. hepatica* infection because of their intra-membranous localisation (bile duct membrane), did not reveal canalicular involvement as described previously by [30] in Zebu West Africa, although hyperplased at some places in the hepatic parenchyma. On the other hand, the presence of artifacts assimilated to cellular debris showing liver fluke trapped and destroyed in situ consolidates our results, even if we were inspired by a single clinical case that proved positive (strongly infested). To clarify this, we analysed the individual values of all animals and found that the percentage of the anti-f2 antibodies to *F. hepatica* of the majority, i.e., the heavily infected animals, was not compatible with the  $\gamma$ GT levels. As examples, we report the values for farms: C6 (202.14% –11.88 IU/l), D3 (282.26% –29.69 IU/l), E7 (54.79% –8.48 IU/l), G2



**Fig. 8** (a 40×) Bile duct hyperplasia surrounded by a thick layer of fibrous connective tissue, (b 10×), (c, d, e 40×). Severe fibrosis of the bile duct, with encapsulated and degenerating immature fluke suspicious of an acellular structure, suggesting calcified *Fasciola hepatica* (f 10×, g 20×, h 40×, i 100×). *HL* hepatic lobules, *CV* centrolobular vein, *PS* periportal space, *PV* portal vein, *TR* trays of remak, *S*

sinusoids, *BC* biliary canal, *FT* fibrous tissue, *GC* glisson capsule, *IC* inflammatory cells, *EPG* eosinophilic polynuclear granulocytes, *IG* inflammatory granuloma, *NPG* neutrophil polynuclear granulocytes, *PT* portal triad, *H* hepatocyte, *HA* hepatic artery, *AS* acellular structure, *D* debris, *DLF* degenerated liver fluke, *RC* red cells

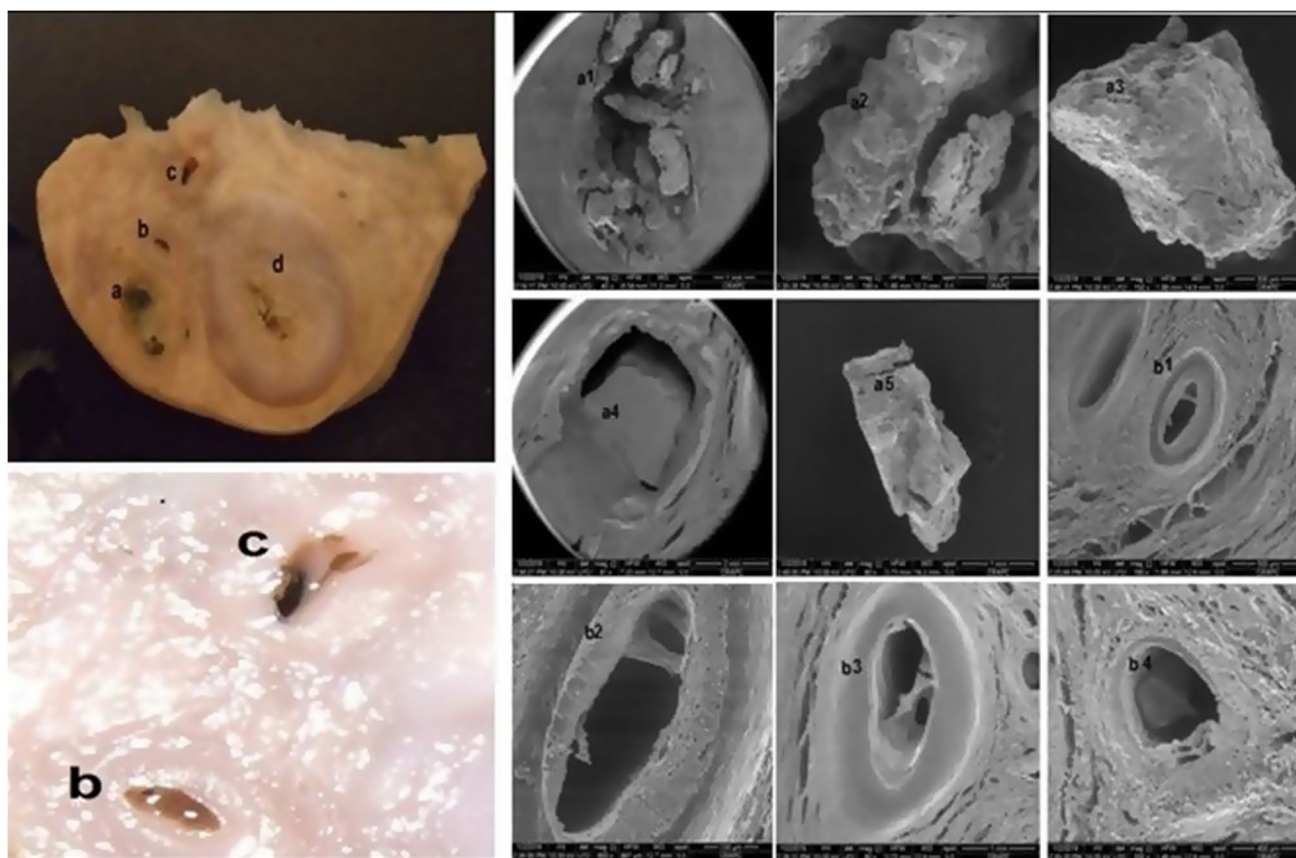
(127.77%–10.6 IU/l), II (182.1%–11.24 IU/l), J1 (141.37%–9.97 IU/l), K7 (88.01%–16.69 IU/l), L15 (165.2%–12.73 IU/l), and M2 (33.28%–7 IU/l), each time one or more cases confirming our hypothesis, namely, the non-specificity of  $\gamma$ GT as an indicator of liver damage.

Although the measurement of the enzymatic activity of transaminases in ruminants is not specific to the liver, transaminases show a fairly high catalytic concentration in this organ. As a result, the insignificant mean levels of AST and ALT found in our study indicate chronic fasciolosis, although this implication contradicted by the work of [6, 10], who report cell destruction and increased membrane permeability to enzymes (cytolysis) during larval migration into the hepatic parenchyma (see also [19]).

## Conclusions

Based on immuno-enzymatic (anti-f2 antibodies of *F. hepatica*), coprological and biochemical tests, we were able to determine the infectivity of the study zone and circumscribe it as the focus of fasciolosis. In addition, our study evaluated the hepatocellular integrity and the metabolic function of the liver. Evaluation of the serological importance of the liver enzymes confirmed the installation of the chronicity during pathology and discarded a hepatic insufficiency relative to both hyperproteinemia and albuminemia. The monitoring of PAL,  $\gamma$ GT, AST, and ALT data also provided some insights, resulting in the





**Fig. 9** Left, macroscopic picture of *F. hepatica* debris in the biliary canal (a, b, c and d) showing liver injuries. Right, details of pathological calcifications (Mag 45×, 180×, 150×, 41×, 80×), observed

via scanning electron microscopy (Fig. 9a). Bar lengths represent: a1: 1 mm, a2: 500 μm, a3: 500 μm, a4: 2 mm, a5: 1 mm, b1: 500 μm, b2: 100 μm, b3: 1 mm, b4: 400 μm

non-establishment of cholestasis and cytolysis. The few variables (age, sex, and breed) introduced into our experimental model, treated independently and in combination, greatly influenced the response of animals to *F. hepatica* infection, albeit to varying degrees, which justifies their importance in the expression of the pathology.

### Compliance with ethical standards

**Conflict of interest** There is no conflict of interest between co-authors and another institution

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