

# Serological Prevalence of Ovine Fasciolosis at Two Slaughterhouse in the Province of Djelfa in Algeria

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## RESEARCH ARTICLE

### Abstract

The present study aims to evaluate the prevalence and some associated risk factors of *Fasciola hepatica* in sheep in the Algerian agro-pastoral region, Djelfa. For this, a total of 217 blood samples were taken from sheep (100 females and 117 males) aged on average ten months in two slaughterhouses in an Algerian agro-pastoral region, Djelfa during the period from January to October 2021 and analyzed by the ELISA method (BIOK 211-Monoscreen AbELISA *Fasciola hepatica* test) at the regional veterinary laboratory of Laghouat. The collected sera were numbered and stored at -20°C until analysis. Technical sheets were filled out with each sampled animal, including information on age, sex, locality and season. The results showed that the rate of positive seroprevalence of *Fasciola hepatica* was 0.46% in sheep. This positivity rate was found in April in a 2-year-old female. At the same time, two doubtful cases (0.92%) were identified, one in a five-year-old female during the month of August and the other in a six-month-old male during the month of September. These results indicate the possibility of fasciolosis infestation in sheep farms in the Djelfa region and the zoonotic risk that this represents, as well as the economic losses that result from it.

**Keywords:** ELISA; *Fasciola hepatica*; seroprevalence; risk factors; sheep; slaughterhouse; Djelfa; Algeria

## INTRODUCTION

Fasciolosis is a zoonotic foodborne disease caused by two trematodes of the genus *Fasciola* in the family of *Fasciolidae*. *Fasciola hepatica* occurs mainly in Europe, Asia, Africa, America and Oceania. *Fasciola gigantica* has been reported in Asia and in Africa (Ashrafi et al., 2014). In some countries with tropical and temperate zones, both *Fasciola* species exist counting Japan, Philippines, Vietnam, Korea, China, India and East Africa (Piedrafita et al., 2010). *Fasciola hepatica* has an indirect life cycle that involves intermediate hosts: *Lymneid* snail; the most common of which is *Galba truncatula* in Europe (Beesley et al., 2018). The definitive host becomes infected by ingesting encysted metacercariae on aquatic vegetation. Each metacercaria releases an immature fluke that will enter the small intestine and migrate through the liver parenchyma where it develops into an adult and produces eggs (Ahmad-Najib et al., 2021). Fascioliasis is a major cause of economic loss for the agricultural industry in both developed and developing countries (Moazeni et al., 2012). It leads to a drop in production (growth, quantity and quality of milk, etc.) (Chauvin et al., 2007). It contributes to annual losses in the livestock industry in North and South America of approximately 2 billion \$ (Nyindo and Lukumbagire, 2015). Moreover, it is also an emerging and re-emerging parasitic disease that has a significant impact on human health

Received: 24 November 2022

Accepted: 05 March 2023

Published: 15 May 2023

DOI:

10.15835/buasvmcn-vm:2022.0032



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worldwide (Mas-Coma, 2005). Andean countries (Bolivia, Peru, Chile and Ecuador), the Caribbean (Cuba), North Africa (Egypt) and Western Europe (Spain) all have major human health problems caused by fasciolosis (Valero et al., 2012).

The diagnosis of fasciolosis is based on post-mortem inspection methods in slaughterhouses by looking for adult forms in the liver. In addition, it is done so by looking for eggs in feces in research laboratories. However, currently available serological techniques can detect *Fasciola* antibodies in serum (Sarkari and Khabisi, 2017). These serological approaches have the advantage of being easy to automate, which is particularly useful when dealing with large sample volumes (Mezo et al., 2011). They give reliable and early results.

In Algeria, there are few studies of this type to search for *Fasciola hepatica* by serological screening. In general, they were based on the search for adult flukes in the liver of animals slaughtered in Algerian slaughterhouses or on the search of specific eggs in feces samples. Among the studies on fasciolosis carried out in Algeria, we cite that of Hamiroune et al. (2020) who reported prevalences of 1.2% and 0.0% respectively in cattle and sheep during an epidemiological survey at the Jijel slaughterhouse during the period from March 2017 to February 2018. Gherroucha et al. (2021) for their part noted in 2018 by a retrospective study carried out from the registers of Constantine slaughterhouses an average prevalence of 2.0% and 0.3% in cattle and sheep. In parallel, Ouchene-Khelifi et al. (2012) found a prevalence of bovine fascioliasis of  $26.7 \pm 2.5\%$  and ovine of  $6.5 \pm 0.4\%$  at the slaughterhouse of El Tarf (Algeria). Similarly, Boucheikhchoukh et al. (2012) found that bovine fascioliasis predominated with an infestation rate of 52.4% during an epidemiological survey of the main bovine helminthoses at the slaughterhouses of Ain El Assel and Bouteldja in the region of El Tarf (Algeria). Moreover, that of Aissi et al. (2009) during a preliminary study on the prevalence of fascioliasis due to *Fasciola hepatica* in some cattle farms in northern central Algeria (Mitidja) and which revealed by serological screening the existence of fascioliasis in 8 localities out of 10 studied, with an average prevalence of 18.8%. Finally, the results obtained in Tissemsilt, Souk-Ahras, Béjaïa, Chemini and Kherrata with prevalence rates are 2.3%; 22.0%, 5.8%, 3.2% and 11.5% respectively (Chougar et al., 2019).

The objectives of this study are, on the one hand, the evaluation of the seroprevalence rate of *Fasciola hepatica* fasciolosis in sheep by the Monocreen AbELISA serological test in an Algerian agropastoral region (Djelfa), and on the other hand, the estimation of certain risk factors involved in the incidence of this disease on the liver as organs of choice widely consumed, cooked by frying or in the form of grilled meats in Algeria, especially in restaurants where the heat treatment may be insufficient.

## MATERIALS AND METHODS

### Presentation of the study area

This study is carried out in Djelfa. This region is located in the heart of the steppe space, and constitutes a transition zone between the high steppe plains of the Tell Atlas and the desert beginnings of the Saharan Atlas. It is bounded by: the wilaya of Médéa to the north, the wilaya of M'sila to the north-east, the wilaya of Tiaret to the north-west, to the east by the wilaya of Biskra, to the south-west by the wilaya of Laghouat and to the South-East by the wilaya of Ouargla. Their following extreme geographical coordinates: 33° and 35° North latitude and 2° and 5° East longitude (Figure 1) (Sba and Benrima, 2017). It is characterized by a contrasting Mediterranean-type climate with a long dry and hot summer season and a rainy and cold winter season (Boussaid et al., 2012).

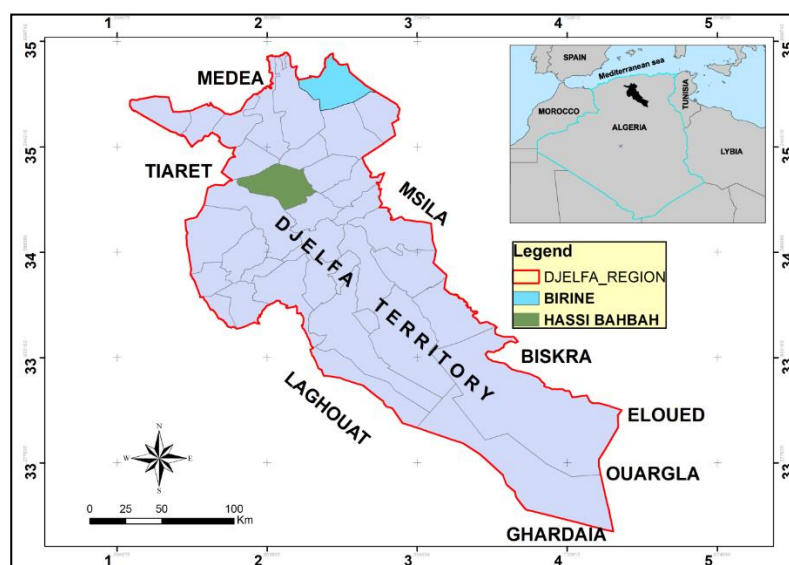


Figure 1. Djelfa province map

The Algerian steppe conceals in its recesses a diversity of plant and animal biological resources. Rich in herbaceous stratum, it thus constitutes an area with a pastoral vocation and a veritable cradle of sheep farming.

No one can dispute the importance of sheep farming in the steppe environment. It is a profitable resource of first choice for breeders in the region.

Livestock systems are thus moving from a pastoral-type mode to an agro-pastoral-type mode and food management is becoming more widespread through rangeland supplementation (Yabrir et al., 2015).

The wilaya of Djelfa is characterized by an immense wealth of sheep. Where 3393000 head were counted in the 2017/2018 campaign, 3456000 head in the 2018/2019 campaign, 4020300 head in the 2019/2020 campaign, 4142800 head in the 2020/2021 campaign and 3353800 heads during the 2021/2022 campaign (DSA, 2023).

The Hassi Bahbah and Birine slaughterhouses are two public establishments among the slaughterhouses located in the Djelfa region, specializing in the production of red meat. The slaughter capacities are different. The Hassi Bahbah slaughterhouse is located about 53 km north of the provincial capital of Djelfa. It operates every day of the week except Friday. It is characterized by the daily slaughter of a large number of sheep and cows. At the same time, the Birine slaughterhouse is located about 135 km from the state capital of Djelfa. It is characterized by a lower slaughter rate compared to the Hassi Bahbah slaughterhouse. The two slaughterhouses are a source of income for many farmers and families in each region, and they contribute to the development of the local and national economy.

### Animals and study period

A total of 217 sheep were sampled before slaughter for serological detection by ELISA of antibodies secreted against *Fasciola hepatica* infestation. These animals were subjected to ante-mortem and post-mortem examination in two slaughterhouses in Djelfa (Birine and Hassi Bahbah) during the period from January to October 2021.

### Risk factors

In order to study the influence of potential risk factors involved in the occurrence of fascioliasis, data on the sampled animals (age and sex of the animals, locality, season and period of study) were recorded at the time of sampling.

### Collection of blood samples

Blood samples (5 ml) were obtained from the jugular vein of animals and labeled, then taken to the regional veterinary laboratory in Laghouat. Blood was centrifuged at 3000 rpm for 15 min and sera were separated and stored at -20°C until used for antibody detection.

### Serological protocol

The commercially available ELISA kit (BIOK 211-Monoscreen AbELISA *Fasciola hepatica* test) (Bio-X Diagnostics- Belgium) was used to check the specificity of the test. The sera were diluted 1/100.

The samples were distributed at a rate of 100 µl per well. The microplate was covered and incubated at 21°C +/- 3°C for one hour. Then, it was rinsed with a washing solution to remove its contents, then drained upside down on a sheet of paper towel. Subsequently, 300 µl of the washing solution were added, then the microplate was emptied again, the washing was repeated twice. The conjugate was diluted 1/50 in the dilution buffer and distributed at the rate of 100 µl per well. The microplate was covered and incubated and the whole at 21°C +/- 3°C for 1 hour, then another wash was applied. The developer was distributed at a rate of 100µl per well and incubated for 10 min at 21°C +/- 3°C in the dark and without covering. Then, 50 µl stop solution was added. The color changes from blue to yellow. Finally, the optical densities were recorded by spectrophotometer using a 450 nm filter. Results should be recorded as soon as possible after application of the Stop Solution.

The result obtained was expressed as a percentage (%) by dividing each value obtained by the corresponding value obtained with the positive serum and multiplied by 100:

$$\text{Values (\%)} = \frac{\text{Delta OD sample}}{\text{Delta OD pos}} \times 100$$

The result is considered positive (+) if the value obtained is greater than or equal to 15%, but less than 45%.

The result is considered positive (++) if the value obtained is greater than or equal to 45%, but less than 75%.

The result is considered positive (+++) if the value obtained is greater than or equal to 75%.

The result is considered doubtful (+/-) if the value obtained is greater than or equal to 10%, but less than 15%.

The result is considered negative (0) if the value obtained is less than 10%.

### Data processing

Serological prevalence was calculated by locality, sex, animal age, season and study period. The sex, season and age were used as a source of variation for risk factors. Confidence intervals (95%) were calculated for the evolution of positive, doubtful and negative seroprevalences. Calculations were performed using Microsoft Office Excel® 2007 software.

## RESULTS AND DISCUSSIONS

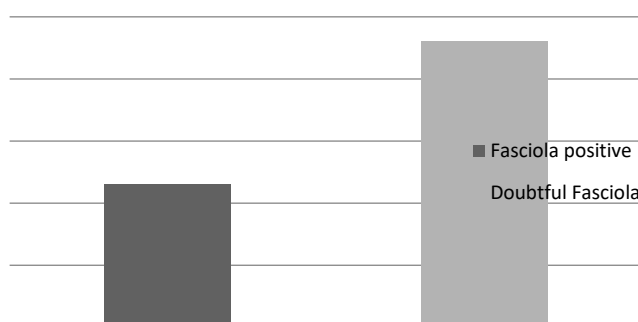
### Global *Fasciola hepatica* seroprevalence results

Of a total of 217 blood serum samples, 0.46% of the samples were seropositive, 0.92% were doubtful, while 98.62% were negative (Table 1, Figure 2). In parallel, the positive seroprevalence rate was recorded during the month of April 2021. While doubtful cases were found during the months of August and September.

**Table 1.** Distribution of the global *Fasciola hepatica* seroprevalence results

Serological results	Number of samples	Prevalence (%)	CI (95%)
Positive	1	0.46	[-0.44 ; 1.36]
Doubtful	2	0.92	[-0.35 ; 2.19]
Negative	214	98.62	[97.07 ; 100.17]
Total	217	100	

CI (95%): 95% confidence interval.



**Figure 2.** Seroprevalence of fasciolosis in sheep in two departments of Djelfa by the ELISA method (BIOK 211-Monoscreen AbELISA *Fasciola hepatica* test).

### Distribution of the seroprevalence rate of *Fasciola hepatica* in sheep according to the study localities

According to the obtained results and which are presented in Table 2, we note that the highest positive seroprevalence was found in the slaughterhouse of Birine (1.54%). On the other hand, no case was detected in the Hassi Bahbah slaughterhouse (0%). In addition, two doubtful cases were recorded in the Hassi Bahbah slaughterhouse (1.32%) (Table 2).

**Table 2.** Distribution of *Fasciola hepatica* seroprevalence rates in sheep according to the study localities

	Localitie A (Hassi Bahbah slaughterhouse)	Localitie B (Birine slaughterhouse)	Total	CI (95%)
Number of animals examined	152	65	217	
Serological results	Positive n(%)	0 (0%)	1 (1.54%)	1 (0.46%) [-0.44 ; 1.36]
	Doubtful n(%)	2 (1.32%)	0 (0%)	2 (0.92%) [-0.35 ; 2.19]
	Negative n(%)	150 (98.68%)	64 (98.46%)	214 (98.62%) [97.07 ; 100.17]

CI (95%): 95% confidence interval.

### Influence of sex on seroprevalence of *Fasciola hepatica* in sheep

During the study period, the highest positive seroprevalence was observed in females with a rate of (1%) compared to males (0%). In parallel, one doubtful case was found in females (1%) and the other in males (0.85%) (Table 3).

According to the serological results, we note that the females were more infested than the males but the same result was obtained for the doubtful of serological results (Table 3).

**Table 3.** Distribution of *Fasciola hepatica* seroprevalence rates in sheep according to sex

	Female	Male	Total	CI (95%)	
<b>Number of animals examined</b>	100	117	217		
<b>Serological results</b>	<b>Positive n(%)</b>	1 (1%)	0 (0%)	1 (0.46%)	[-0.44 ; 1.36]
	<b>Doubtful n(%)</b>	1 (1%)	1 (0.85%)	2 (0.92%)	[-0.35 ; 2.19]
	<b>Negative n(%)</b>	98 (98%)	116 (99.15%)	214 (98.62%)	[97.07 ; 100.17]

CI (95%): 95% confidence interval.

### Effect of age on the seroprevalence of *Fasciola hepatica* in sheep

The high positive seroprevalence rate (0.81%) was found in 2-year-old sheep. While for the other age group, no cases were detected (0%). In addition, for doubtful cases, two cases were recorded, one in sheep aged less than two years (six months) (0.81%) and the other case in sheep aged five years (1.08%) (Table 4).

**Table 4.** Distribution of *Fasciola hepatica* seroprevalence rates in sheep according to age groups

	≤ 2 years	>2years	Total	CI (95%)	
<b>Number of animals examined</b>	124	93	217		
<b>Serological results</b>	<b>Positive n(%)</b>	1 (0.81%)	0 (0%)	1 (0.46%)	[-0.44 ; 1.36]
	<b>Doubtful n(%)</b>	1 (0.81%)	1(1.08%)	2 (0.92%)	[-0.35 ; 2.19]
	<b>Negative n(%)</b>	122 (98.39%)	92 (98.92%)	214 (98.62%)	[97.07 ; 100.17]

CI (95%): 95% confidence interval.

### Effect of season on the seroprevalence of *Fasciola hepatica* in sheep

In the light of the results obtained, it appears that in sheep, the high rate of positive seroprevalence was observed during the spring season (2.17%). While the doubtful seroprevalence rate was reported during the summer season (1.85%) (Table 5).

**Table 5.** Distribution of *Fasciola hepatica* seroprevalence rates in sheep according to season

	Winter	Spring	Summer	Autumn	Total	CI (95%)	
<b>Number of animals examined</b>	11	46	108	52	217		
<b>Serological results</b>	<b>Positive n(%)</b>	0 (0%)	1 (2.17)	0 (0%)	0 (0%)	1 (0.46%)	[-0.44 ; 1.36]
	<b>Doubtful n(%)</b>	0 (0%)	0 (0%)	2 (1.85%)	0(0%)	2 (0.92%)	[-0.35 ; 2.19]
	<b>Negative n(%)</b>	11 (100%)	45 (97.83%)	106 (98.15%)	52 (100%)	214 (98.62%)	[97.07 ; 100.17]

CI (95%): 95% confidence interval.

The main objective of this study was to evaluate the seroprevalence rate of *Fasciola hepatica* fasciolosis in sheep in the region of Djelfa (Algeria) and to investigate the involvement of certain risk factors in the disease.

Fascioliasis is an indirect parasitic zoonosis in which humans and animals are infested by metacercariae found on damp or submerged plants. Moreover, it is a pathology with mandatory research in Algerian slaughterhouses where suspected cases are highlighted by post-mortem inspection (visual, palpation and incision) of the livers. For this, the ELISA is considered as a method of choice and rapid for the early confirmation of fascioliasis before installation of cholangitis and adult flukes in the bile ducts.

It should be noted that this study is considered as the first of its kind in the agro-pastoral region of Djelfa during which the indirect ELISA method (BIOK 211-Monoscreen AbELISA *Fasciola hepatica* test) was used to determine the seroprevalence of *Fasciola hepatica*.

In general, our results show that sheep are the only ones affected by *Fasciola hepatica* fascioliasis with a seroprevalence rate of 0.46%. In addition, two doubtful cases were recorded in sheep. These results can be explained by the involvement of several factors. In this sense, it should be noted that sheep raised in the open and feed on grass in contact with the ground, which promotes the transmission of fascioliasis (Hamiroune et al., 2019).

The distribution of the results according to the study localities showed that the high positive seroprevalence rate (1.54%) was found in the Birine slaughterhouse. In addition, two doubtful cases were observed in the other locality (1.32%). In parallel, the positive rate was recorded during the month of April 2021 (the spring). According to Merdas Ferhati (2015), the lack or absence of any treatment through control campaigns and an effective and serious vaccination strategy before the arrival of the seasons (spring-autumn) would favor the appearance of the condition and the increase in prevalence during these two seasons.

Our results showed that in sheep, the positive seroprevalence rate of our study (0.46%) is close to those reported by Rasheed and Kadir (2008), in the region of Kirkuk in Iraq with a prevalence rate of 0.50%. On the other hand, it remains significantly lower than the results reported by Yaşar Çelik and Aslan Çelik (2018) in a similar study with a seroprevalence rate of 7.50% in sheep and those of Afshan et al. (2013) in Pakistan with a rate seroprevalence of 39.2%. As well as those of Hammami et al. (2007) in Tunisia and Ahmed et al. (2007) in Ethiopia, who found rates of 35% and 13.5%, respectively. Similarly, it remains lower than that found by Bendiaf (2011) in Constantine (Algeria) in a similar study with a seroprevalence rate of 18.91% in sheep. Moreover, it is also lower than the result reported by Mekroud et al. (2004) in Jijel (Algeria) and Constantine (Algeria) who found seroprevalence results of natural *Fasciola hepatica* infections in sheep of 23.5% and 6.4%, respectively. The low seroprevalence rate of our result can be explained also by differences in temperature, humidity, and soil that could favor the multiplication of the intermediate host (Bitew et al., 2010) and the excessive use of antihelminthiasis drugs, as well as the structure of the herd which is characterized by batches of animals raised on dry pasture. In addition, the Djelfa region is characterized by unfavorable meteorological and environmental conditions for the survival of the intermediate host (*Limnée*). It is characterized by a markedly semi-arid to arid climate with a continental nuance. Indeed, it is semi-arid in the areas located in the central and northern parts of the wilaya and arid in the entire area located in the southern part of the wilaya (ANDI, 2013). Similarly, the place where the animals are raised, the biotopes, the climate, the age or even the absence of an integrated pest management program play a role in the appearance of the disease (Boucheikhchoukh et al., 2012). In addition, the natural vegetation cover of the wilaya of Djelfa consists essentially of high arid steppes with voids between the tufts of vegetation on generally poor soils in direct contact with the bedrock. Djelfa is globally part of the alfa steppe. This perennial grass occupies a large part of the territory of the wilaya, in particular the area of the southern pre-desert plateau (ANDI, 2013). At the same time, livestock farming in the Djelfa region is characterized by a certain monotony, regardless of the bioclimatic level. The breeding system is random, anarchic in a way. Breeders tend to settle down. Grazing is common practice. The use of concentrate supplementation is mostly used (Yabrir et al., 2015).

The distribution of the results according to sex showed that the positive seroprevalence rate of 1% was observed in a female sheep and no cases were recorded in males. Similarly, two cases were found, one in a female and the other in a male. These results are in agreement with those of Bitew et al. (2010). On the other hand, they are different from those found by Ahmed et al. (2007), who found a high prevalence in males compared to females. Our results can be explained by the effect of several factors, in particular, during the fattening period, in the majority of cases, the animals (mainly the males) prepared to be sent for slaughter, are reared in an intensive system and are returned to a closed environment and fed on dry fattening foods such as straw, hay, corn, bran and other concentrates; the latter are less exposed to the infesting form than natural grass. In addition, depending on the ability of the breeders and the veterinary practitioners who follow the breeding, these animals are treated with antiparasitics against parasites and with antibiotics against infectious diseases, as well as other drugs such as vitamins, which leads to a decrease in the number of cases of parasitized organs despite the presence of humidity in the external environment (Hamiroune et al., 2020).

According to the age groups, the highest positive seroprevalence (0.81%) was found in a two-year-old female, with two doubtful cases, one in a five-year-old female and the other in a male under two years (six months). It should be noted that age is also an important factor for the prevalence of fascioliasis in ruminants. The results of the present study are not consistent with those of Afshan et al. (2013). In fact, the authors reported that fascioliasis is more common in older animals than in younger animals and that could be due to greater exposure of older animals to the parasitic stages and its intermediate host and that younger animals are not allowed to go out to graze. Additionally, the higher exposure risk of adults may be due to physiological differences, such as stress, pregnancy, lambing, inadequate diet and infectious diseases (Ahmed et al., 2007). On the other hand, other authors say that the infestations are stronger and the disorders are more serious in young people and that acquired immunity sets in with age on the one hand, and contact with the parasite on the other hand. In addition, ruminants develop resistance

to pathogens linked to repeated infestations with age (Doyle, 1972). The information drawn from the work of these authors may explain the low percentage recorded during our present study.

Depending of the study seasons, our results showed that the positive seroprevalence rate (2.17%) was observed during the spring season (month of April). On the other hand, Ouchene-Khelifi et al. (2018) were shown that the highest prevalence rate was observed during the winter season (9.2%). Moreover, in Tunisia, Jemli et al. (1991) reported that fascioliasis mainly occurs during the summer season. Anthelmintics have been used for the treatment of fascioliasis, particularly in sheep, during two periods of the year (October-November and March-April) which explains the low prevalence of fascioliasis in sheep. Similar results were observed by Ullah et al. (2016). In addition, the Djelfa region is characterized by a cold and harsh winter and a hot and dry summer, hence the limitation of the activity of vegetative life. Precipitation is low and variable from one year to another in terms of quantity and distribution. The thermal regimes are relatively homogeneous and reflect a continental-type climate (Boussaid et al., 2012).

Finally, the ELISA (BIOK 211-Monoscreen AbELISA *Fasciola hepatica* test), remains a priority procedure to perform in sheep farms for the early detection of *Fasciola hepatica* and practice an effective treatment before the installation of adult flukes in the bile ducts. which causes significant economic losses and poses a major health risk to consumers in the future. According to Chauvin et al. (2007), in farm animals, fasciolosis leads to a drop in production (growth, quantity and quality of milk, etc.), a reduction in traction capacity in buffalo, seizures of livers in slaughterhouses and sometimes the mortality of infested animals (especially in sheep infested with *Fasciola hepatica*).

## CONCLUSIONS

This study is considered as the first of its kind in the agro-pastoral region of Djelfa during which the indirect ELISA method (BIOK 211-Monoscreen AbELISA *Fasciola hepatica* test) was used to determine the seroprevalence of *Fasciola hepatica* in sheep.

The results of this study confirm the importance of using the ELISA method in the early detection of *Fasciola hepatica* infestation. Moreover, it is a diagnostic method of choice in the epidemiological context of the Djelfa region.

Our results showed that the overall rate of positive seroprevalence for *Fasciola hepatica* was 0.46% during the month of April in a 2-year-old sheep; With a doubtful seroprevalence rate of 0.92%.

The results suggest that the indirect ELISA is a technique for the early diagnosis of the infection and another way to better understand the epidemiology of fascioliasis as the disease decreases in the region, but it should be kept in mind that 'he still exists. Moreover, they are considered as an important indicator of the lack of preventive and curative treatment of animal species by practicing veterinarians. For this, it is important to act on the life cycle of the pathogen causing this disease in order to control the transmission of the causal agent upstream and to call for the continued application of control and prevention measures. In addition, sensibilization, and popularization of breeders, consumers and other actors in the food chain is mandatory. In addition, it will be very useful to carry out systematic screening in all farms in the country by general and specific laboratory examinations for confirmation. All this will contribute to better fight against the disease but would also make it possible to limit imports of meat.

**Author Contributions:** S.H. Work in the slaughterhouse and in the laboratory and writing of the article; M.H. Design, planning and article writing; R.S. Supervision, revision and article writing.

**Funding Source:** This research received no external funding.

## Acknowledgments

The authors thank all the staff of the Hassi Bahbah and Birin slaughterhouse in Djelfa, Algeria (The Veterinary Doctors, the Veterinary Inspector, and all the workers). As well as all the members of the regional veterinary laboratory of Laghouat.

## Conflicts of Interest

The authors declared no conflict of interest.

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