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Cross-sectional Study on Ruminant Tuberculosis in the Province of Bouira, Algeria

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Abstract
Tuberculosis (TB) is a chronic infectious disease. In Algeria, it is recognized as endemic in ruminants. A prospective study was conducted in two slaughterhouses in the province of Bouira, Algeria from June to September, 2019 to estimate the prevalence of tuberculosis in cattle and sheep, as well as the associated risk factors. A total of 2623 ruminant carcasses (1059 cattle and 1564 sheep) were inspected. The apparent prevalence of the disease based on the visible lesions in ruminants was 35/2623 (1.33%), representing 26/1059 (2.45%) and 09/1564 (0.57%) from cattle and sheep respectively. A significant difference between the two slaughterhouses was P< 0001.Crossbreed (57.69%) are the most affected by the disease. In the current study, males (100%) were the only ones affected by tuberculosis lesions, while cattle (1 year > -2 years) (69.23%) were the most affected. A microscopic examination of 26 samples revealed that 3(11.54%) harbored acid-fast-bacilli (AFB). The culture test revealed that a total of 12(46.15%) tubes were positive and 14(53.85%) were negative. A significant difference in tubercular lesions was observed between sheep breeds with the local breed was the most affected (66.67%), P<0.0001. Females were more affected (55.56%) than males (44.44%); (P = 0.1610). the cull sheep were the most affected by TB lesions (44.44%). Microscopy examination was negative in sheep lesions and the culture has shown that only 1/9 (11.11%) was positive while 8/9 (88.88%) were negative. Granulomatous inflammation has been observed in most tuberculous lesions. Finally, additional studies are necessary in order to know the real situation of tuberculosis in Algeria.

Keywords
Bouira, Culture, Microscopy, Slaughterhouses, Tuberculosis

1. Introduction
Bovine tuberculosis (BTB) is a chronic bacterial disease caused by *Mycobacterium bovis* (*M. bovis*); a member of a group known as *Mycobacterium tuberculosis* complex (MTC). It predominantly affects cattle but also other domesticated species **(More et al., 2017).** Cattle and buffalo are considered to be the maintenance hosts for *M. bovis* **(Hlokwe et al., 2014).** Bovine species including bison and buffaloes are susceptible to the disease, but nearly all warm-blooded animals can be affected including sheep, goats, horses, pigs, deer, antelope, dogs, cats, ferrets, camels, foxes, mink, badgers, rats, primates, llamas, kudus, elands, tapirs, elk, elephants, sitatungas, oryxes, addaxes, rhinoceroses, opossums, ground squirrels, otters, seals, hares, moles, raccoons, coyotes, lions, tigers, leopards, and lynx **(De Lisle et al., 2001; Oíreilly and Daborn, 1995).** Early infections of this disease are often asymptomatic. The clinical signs vary; lung involvement may be manifested by a cough, Dyspnea and other signs of low-grade pneumonia **(Garner, 2004).** The diagnosis is often made after slaughter by several exams. A *post-mortem* examination should be carried out on all the tissues of the body but the lesions are most often observed in the lymph nodes (particularly of the head and thorax), lungs, intestines, liver, spleen, pleura, and peritoneum **(Teklu et al., 2004).** However, histopathological examination is one of the official methods recognized by the European Union, but their

specificity is low (estimated at 92.3%) **(Varello et al., 2008).** On the other hand, their sensitivity is high, at around 93% in a population of animals with lesions **(Courcoul et al., 2014).** *M. bovis* detection continues to be an important tool in the early diagnosis of the disease, determining its prevalence and eco-epidemiological status, and thus contributing to its eradication **(Livingstone et al., 2015).** is The real prevalence of ruminant tuberculosis is still unknown in Algeria and this is due to the absence of credible scientific information concerning this disease, despite several studies having been carried out. On the other hand, this zoonosis remains a major scourge which has decimated a large number of ruminants and is responsible for huge losses of meat and offal in slaughterhouses. Because of Tuberculosis is a major zoonosis and is the cause of direct and indirect economic losses **(Crozet et al., 2020)** an increase in research is required in order to obtain a bigger picture of ruminant TB prevalence areas. For this, a cross-sectional survey on cattle and sheep was carried out in two slaughterhouses located in Bouira in order to evaluate the prevalence of ruminant tuberculosis in the study area and diagnose it by bacilloscopy, bacterial culture and histopathological examination.

2. Materials and Methods

2.1. Study Area

This study was conducted during the period between July 22 until September 07, 2019 in the province of Bouira, located in the northern part of Algeria, south-east of the capital. This area is characterized by a semi intensive bovine farming system with variable climatic conditions. The ruminants slaughtered in the two private abattoirs (Oued Housand and Bechloul) are brought from different areas of Bouira. These two slaughterhouses include a room for the slaughter of cattle, sheep and goats. The daily slaughter capacity at the Oued Hous Slaughterhouse is 16 cattle and 18 sheep, while for the Bechloul Slaughterhouse is 18 cattle and 23 Sheep **Fig. (1).**

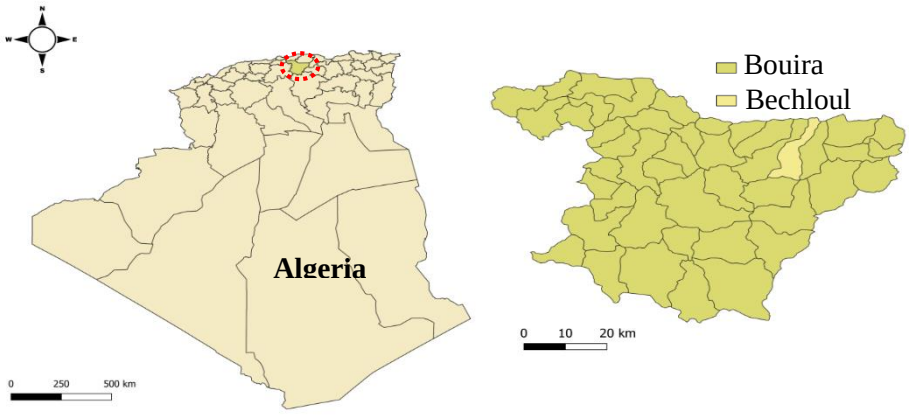


Fig. 1. Study area

2.2. Ethical Approval

The methodology protocols of the present study followed the ethical concepts in animal experimentation, recommended by FELASA and approved by the Algerian Ministry of Higher Education and Scientific Research (Executive Decree 10-90 supplementing the Algerian government decree 04-82) and the AASEA (45/DGLPAG/ DVA.SDA.14).

2.3. Post-mortem Examination

Post-mortem Examination is considered passive surveillance of this disease **(Schiller et al., 2010).** A total of 2623 ruminant carcasses (cattle and sheep) were subjected to a detailed post-mortem investigation; a thorough examination was conducted on the mandibular, parotid, retropharyngeal, bronchial, mediastinal and mesenteric lymph nodes, together with the thoracic and abdominal organs **Fig. (2).** TB suspected tissue samples were collected in sterile bottles and processed according to standard operating procedures for mycobacterial culture. All animal specimens were transported in a cold chain using an ice box packed with ice to the laboratory.

2.4. Microscopy and Culturing Examination

All specimens were processed using standard methods **(Githui, 1993).** Lesions were dissected and homogenized manually then decontaminated with the most traditional method used to isolate *M. bovis* from bovine tissues; the Petroff method in which a 4% NaOH solution is used as a decontaminant **(OIE, 2014).** The sediment of decontaminated samples was used for direct microscopic examination by using the *Ziehl-Neelsen* staining, followed by observation under an optical microscope with an immersion objective (X100), and culture by inoculating on three media slants Lowenstein–Jensen (*LJ*),The slants were incubated at 37°C and observed once weekly for 12 weeks **(OIE, 2010).** Cultures were considered negative if no visible growth was detected after 8 weeks of incubation. Microscopic examination of cultures using the *Ziehl–Neelsen* staining method was performed to detect the presence of acid-fast-positive bacilli (AFB) **(Roberts et al., 1991).**

2.5. Histopathological Analysis

The histopathological analysis was carried out at the level of the anatomical pathology laboratory in the Veterinary

Sciences Institute of Tiaret University, Algeria. Biopsy specimens for histological examination are obtained after *post-mortem* examination. Positive cases' samples of the lung, liver, and lymph nodes were routinely processed by an automatic tissue processor (LeicaTP1020). According to the procedure outlined by **(Suvarna et al., 2018),** the tissues were embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). Using a Zeiss optical microscope with a camera, photomicrographs of selected lesions were taken (AwioCam EPc 5s). Staining slides were examined to assess histological alterations including tuberculous granulomatous inflammation witch was typed according to the occurrence and arrangement of the following main components: caseous necrosis with or without mineralization, epithelioid cells, multinucleated Langhans type giant cells (MNGCsOzturk-Gurgen, lymphocytes and fibrous connective tissue **(Ozturk-Gurgen et al., 2020).** The histological diagnosis makes it possible to highlight the characteristic lesions of the mycobacteria family; it is therefore not specific to *M. bovis* and requires a bacteriological isolation to be able to confirm that these are lesions linked to *M. bovis* and conclude that there is tuberculosis infection.

2.6. Data Analysis

Data from the slaughterhouse and laboratory were entered into a Microsoft Excel spreadsheet, and statistical analysis was carried out with SAS software **(SAS, 2005).**Descriptive statistics were used to analyze the prevalence of tuberculous lesions and the proportion of risk factors (sex, age and breed). Differences were considered significant at $p<0.05$.

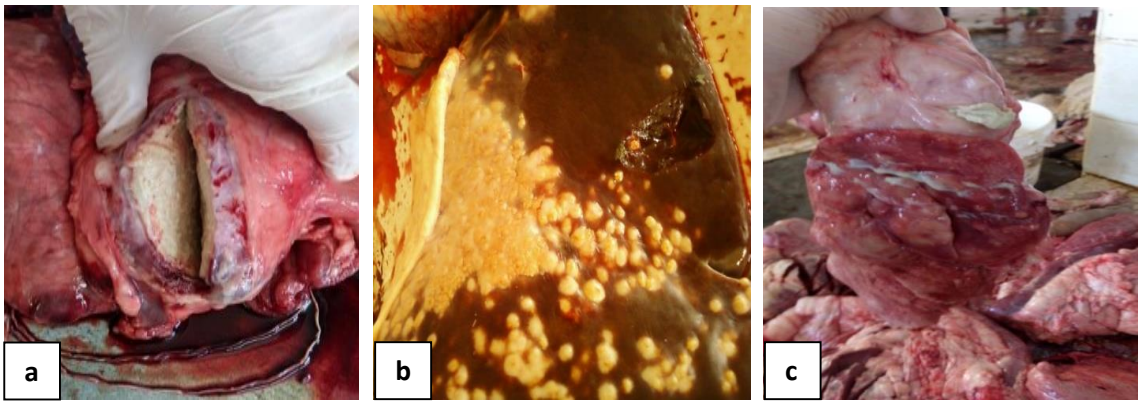


Fig. 2. (a, b, c) organs suspected of tuberculosis lesions, **a.** caseo-calcareous ovine mediastinal lymph node, **b.** Liver lesion (bovine), **c.** Lung lesion (bovine).

3. Results

3.1. Prevalence and Risk Factor of Bovine Tuberculosis

Out of 1059 cattle carcasses subjected to sanitary inspection for the detection of tuberculosis like lesions during the study period, the *post-mortem* inspection showed a prevalence of 2.45% of carcasses with TB-like lesions. Our results show that the slaughterhouse of Bouira indicates the largest number of animals affected by bovine tuberculosis, with a significant difference between the two slaughterhouses ($p< 0.0001$) **(Table, 1).** Bovine tuberculosis risk factor results showed that cross breeds have the highest percentage of tuberculosis lesions (57.69%), followed by the same rate of the local and prim Holstein breeds (15.38%), then the Charolais breed has a rate of (7.69%) and a small percentage was recorded in the Montbeliard breed with ($Pr<= 0.137$), **(Table, 1).** However, cattle whose age groups are between 1-2 years are the most affected by tuberculosis (69.23%), compared to the other age groups **(Table, 2).** The males are the most affected by bovine tuberculosis compared to Our results have clearly shown the presence of acid-alcohol fast bacilli (AFB) **(Fig. 3).** Bacteriological examination from cattle revealed that bacilloscopy indicates a low rate of females, but there is no significant difference between the sexes ($p=0.9514$) **(Table, 1).**

3.2. Prevalence and Risk Factor of Sheep Tuberculosis

A total of 1564 sheep carcasses were inspected in the same establishments. Our results showed a very small (0.57%) proportion of tuberculosis lesions in this species. The tuberculosis lesion in sheep is more important at the Bouira Slaughterhouse (55.56%), compared to the Bechloul slaughterhouse (44.44%); ($P=0.0803$) **(Table, 2).**This disease is significantly more important in the local breed compared to other breeds ($P=0.0001$), **(Table 2),** and the females are more affected than males (55.56% vs. 44.44%) but there is no significant difference ($P=0.1610$), **(Table, 2).** A high proportion is observed in reformed breeds (44.44%) compared to other age classes we noted a significant difference between age groups ($P=0.0361$), **(Table, 2).**

3.3. Bacteriological Examination

positivity (11.54%); and (46.15%) positive cultures were recorded compared to (53.85%) negative **(Table 3, Fig. 3).**

Table 1. Risk factors of Bovine tuberculosis lesions (TBL).

Parameters	TBL (%)	NTBL (%)	p-value
Slaughterhouse			
Bouira	100.00	45.74	< 0001
Bechloul	00.00	54.26	
Breeds			
Cross	57.69	67.73	
Local	15.38	22.19	0.0137
Prim Holstein	15.38	2.91	
Charolais	7.69	6.30	
Montbeliard	3.85	0.87	
Sex			
Male	100.00	99.81	
Female	00.00	0.19	0.9514
Age group			
6 months	3.85	0.00	
1years	3.85	1.07	
1ans> - 2years	69.23	80.33	0.0361
2ans> - 3years	11.54	9.21	
3ans> - 4years	11.54	7.85	
4ans> - 5years	00.00	0.29	
Reforme	00.00	1.26	

TBL: Tuberculosis lesion, **NTBL**: Non Tuberculosis Lesion

Table 2. Distribution of sheep tuberculosis lesions.

Parameters	TBL (%)	NTBL (%)	p-value
Slaughterhouse			
Bouira	55.56	30.72	0.0803
Becheloul	44.44	69.28	
Breeds			
Cross	22.22	30.85	0.0001
Local	66.67	12.15	
Ouled djelal	11.11	57.01	
Sex			
Male	39.27	60.73	0.1610
Female	55.56	44.44	
Age group			
1 year	11.11	00.00	
1ans> - 2years	22.22	19.99	0.0361
2ans> - 3years	00.00	00.90	
4ans> - 5ans	11.11	20.50	
5ans> - 6ans	11.11	06.94	
Reforme	44.44	51.67	

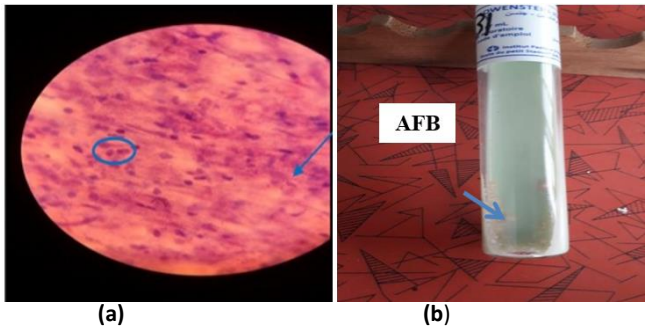
TBL: Tuberculosis lesion, **NTBL**: Non Tuberculosis Lesion.

Table 3. Bacteriological examination

Smear microscopy	Negative (%)	Positive (%)
	88.46	11.54
	Negative (%)	Positive (%)
Culture	53.85	46.15

3.4. Histopathological Findings

The lung samples; Chronic active pleurisy with fibrous thickening and vascular dilatation were marked **(Fig. 1A, B, C).** Concerning the liver tissues they showed localized chronic supurative hepatitis with fibrosis and hepatocellular necrosis. The presence of edema demonstrates a reactivation of the infection **(Fig. 4A, B, C, D).** Regarding the lymph nodes, the histopathological examination revealed lymphadenitis and pyogranuloma; multifocal to coalescing, severe, chronic active, with the presence of multinucleated cells of the pathognomic Langhans type **(Fig. 3A, B, C, D).**



(a) (b)

Fig. 3. (a): AFB Optical Microscope; (b):Positive Culture

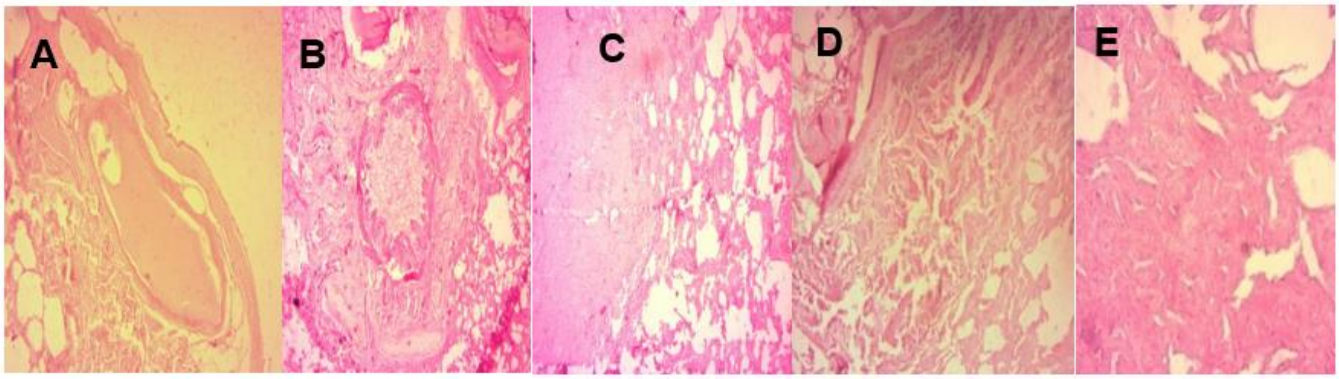


Fig. 4. A (Chronic active pleurisy with fibrous thickening and vascular dilatation. H&E. X 100); **B** (Bronchitis, bronchus containing an exudate composed of a moderate number of degenerated neutrophils, and leukocytes, the peribronchial space is dilated by edema fluid and inflammatory cells (flatus). H&E. X 100. (bovine lung); **C** (On the left a massive fibrosis and on the right an interstitial pneumonia H&E. X 100); **D** (An exudate composed of edema, fibri, cellular debris and inflammatory cells); **E** (Interlobular partitions are enlarged by fibrin with the presence of some fibroblasts and collagen fibers H&E. X 400).

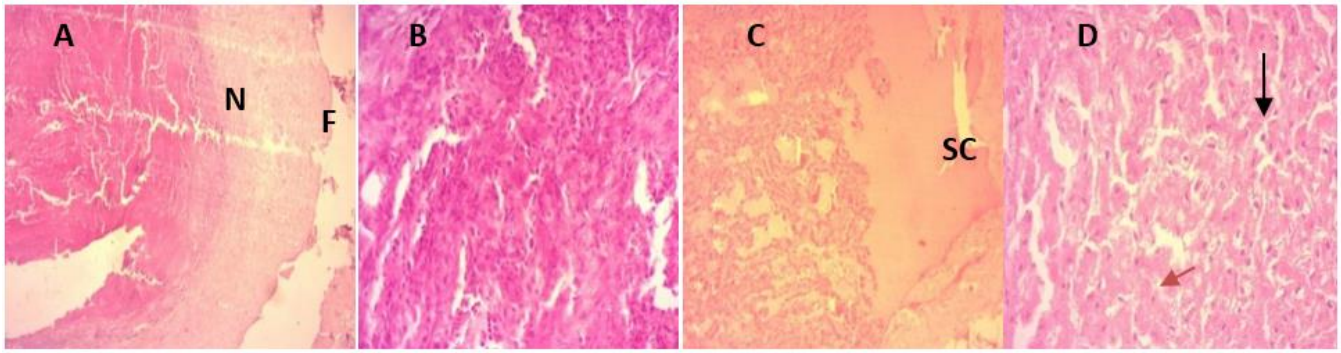


Fig. 5. A (pyogranuloma with a center of necrosis (N) surrouned by a thick layer of fibrosis (F)H&E. X100); **B** (Inflammatory lymphoplasmacytic infiltrate H&E. X 400);**C** (subcapsular (SC and intercellular tissue edema H&E. X 400); **D** (cellular necrosis with cytoplasmic vacuolation, nuclear pyknosis and karyolosis H&E. X 400).

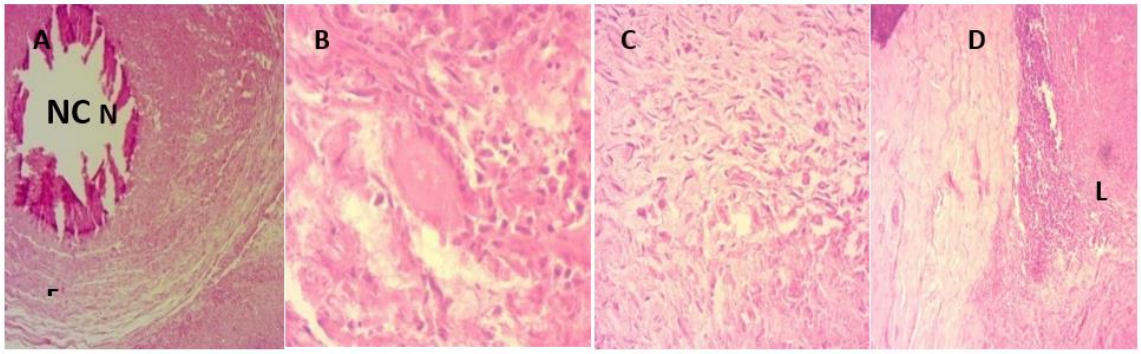


Fig. 6. A (Lymph node a central zone of calcified necrosis (NC), a zone of necrosis consisting of abundant cellular debris (N) and alymphoplasmocytic infiltrates in the fibrous stroma (F), H&E.X100); **B** (langhans-type multinucleated cells (nuclei arranged in a horseshoe), H&E. X 400); **C** (Moderate number of lymphocytes, plasma cells and fibroblasts within fibrous connective tissue H&E. X 400); **D** (persistence in places of clusters of lymphocytes(L) H&E. X 100.

4. Discussion

In Algeria, *post mortem* examinations for suspicious lesions at the slaughterhouse are the official diagnostic procedures for tuberculosis **(Sahraoui et al., 2011).** Tuberculosis lesions are not visible macroscopically until late and can be confused with other infections other than tuberculosis. The absence of a lesion does not prove the absence of infection and if a lesion is found, only a suspicion can be expressed, requiring bacteriological and histological confirmation after sampling **(Corner, 1994; Liebana et al., 2008).** The present study provides interesting data related to bovine and ovine tuberculosis in two slaughterhouses Bouira (Algeria). Our results recorded a lower prevalence (26/1058; 2.46%) of lesions on post-mortem macroscopic inspection of bovine carcasses slaughtered compared to that of **Damene et al., (2020)** (6.5% in 2020) in two provinces (Béjaia and Sétif) in North-Eastern Algeria. This value is also lower than the values (10.13%) reported by **Tazerart et al., (2021)** in a study carried out in five slaughterhouses in in the northern part of Algeria. However, this rate is came in accordance with that reported by **Ayad et al., (2020)** 2.06% (4092/199 077) in a retrospective study of the province of Bejaia (Algeria), and also is comparable to those previously reported in Algeria in 2007 (3.6%) by **Sahraoui et al., (2009)**

and in Morocco between 2014 and 2015 (3.7%) **(Yahyaoui-Azami et al., 2017).** Whereas in some African countries, such as Morocco, Algeria, Sudan, Uganda and Ethiopia, the prevalence of lesions suggestive of gross visible TB was found to be as high as 5.2% **(Shuaib et al., 2018; Ayad et al., 2020).** We recorded a significant difference between the two slaughterhouses (P < 0001). Crossbreed (57.69%) are the most affected by this disease compared to foreign or improved breeds.

In this work, the males (100%) are the only ones affected by lesions tuberculosis than females (0%). It can be explained that most of the slaughtered animals are males and are the most exposed, in accordance with regulations prohibiting the slaughter of female animals other than in cases of necessity **(Teklu, 2004).** Same observation was declared by **Belakhal et al., (2021)** who reported that the rate of infection in males (145/184; 78.8%) is higher than females (39/184; 21.2%). On the other hand, our results are different to **Kardjadj and Yala (2012)** in a study conducted at a slaughterhouse in the central region of Algeria; overall, females were more at risk (31/48; 7.29%) when compared to males (17/48; 1.47%). In the present study, young animals (1years >- 2years) (69.23%) were more frequently infected by TB than older ones. This can be explained by congenital transmission **(Menzies et al., 2012).** Pseudo-vertical transmission (such as ingestion of contaminated colostrum) is often related to close contact between cows and their calves **(Belakehal et al., 2021).**

Our study recorded a very low prevalence of lesion tuberculosis in sheep (09/1565; 0.57%) compared to that of **Sahraoui et al., (2012)** with (4.40%). According to **Hadjab et al., (2019),** the prevalence of ovine tuberculosis lesions was recorded at 1% in a study conducted in the Adrar region in south Algeria. However, the presence of tuberculosis lesions in sheep could indicate contamination following cohabitation or shared grazing with infected cattle **(Pesciaroli et al., 2014).** A significant difference in tubercular lesions was observed between the categories of sheep breeds. The local breed is the most affected (66.67%; P<0.0001). This investigation has shown that females (55.56%) are more affected by tubercular lesions than males (44.44%; P=0.1610). The cull sheep are the most affected by TB lesions compared to other age groups (44.44%). Our results are similar to those reported by **Sahraoui et al., (2012)** and **Kardjadj and Yala (2012).** This finding can be explained by the fact that the prevalence of infection with this disease is positively correlated with the age of the animal and the risk of infection increases in older animals due to prolonged exposure to the pathogen in the environment **(Milne et al., 2020; Dejene et al., 2016).**

Microscopic examination of 26 samples from bovine lesions revealed that 3(11.54%) harbored acid-fast-bacilli (AFB) and 23(88.46%) were negative. These results are unsatisfactory, but this is not surprising as microscopy is not sensitive and it is only positive if the sample contains 5000 to 10000 bacilli/ml **(Proano et al., 2011).** In addition, it should be noted that microscopy is not specific as all mycobacteria are acid-fast **(Carbonelle et al., 2003).** Concerning the culture test from 26 samples, bovine lesions revealed 12(48.15%), tubes were positive and 14 (53.85%) were negative.

Our finding is not consistent with that of **Sahraoui et al., (2008)** who only found 28.85% of positive smears and a culture positivity rate of 51.5%. The results are also different from those found by **Aljameel et al. (2014),** who showed that out of 163 samples analyzed, 124 (76.1%) were smear positive while 39 (23.9%) were Acid-fast bacilli (AFB) negative **(Aljameel et al., 2014).** This difference can be explained by the low number of samples tested in our study. On the other hand, microscopy was negative in sheep lesions. **Sahraoui et al., (2012)** indicated that microscopy is negative in sheep. So the culture of ovine lesions has shown that 1/9 (11.11%) were positive and 8/9(88.88%) were negative. On the other hand, this result is different from those reported by **Sahraoui et al., (2012)** in the same study on sheep. However, this finding is comparable to those reported by **Muñoz-Mendoza et al., (2016)** who stated that the number of reported cases of tuberculosis in sheep has increased recently in Europe and it has even been suggested that sheep could be a reservoir of bovine tuberculosis in Spain. In the present study, the histopathological examination was carried out according to the method of **Mckinleyt et al., (2018).** It is a fast method (the result is obtained in five to seven days). Histopathological examinations of suspected tuberculosis lesions revealed typical granulomas characterized by central necrosis surrounded by a thick layer of fibrosis, epithelioid and lymphocytic cells. Multinucleated Langhans cells are also seen in some samples. The results of the histopathological examination were similar to those of **Aljameel et al., (2014)** and **Whipple et al., (1995)** who observed typical granulomatous lesions in tissues with macroscopic lesions pathognomonic for bovine tuberculosis. Results also similar to those reported by **Mckinleyt et al., (2018)** who indicated that few Langhans-type multinucleated giant cells are displayed. Our findings are consistent with those reported by **Sahraoui et al., (2012)** where lesions in the lungs, liver and lymph nodes showed giant epithelial follicular granulomatous lesions with central caseous necrosis.

5. Conclusion

Results confirmed the presence of tuberculosis lesions in cattle and sheep in the area under investigation however, tuberculosis in sheep exists with a low prevalence. Finally, tuberculosis is still a threat to our farms, which requires a high level of surveillance

6. Conflict of Interest

Authors declare no conflict of interest.

7. Acknowledgements

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