Introduction

In Algeria, livestock production is an important part of agriculture, it account for a significant proportion of gross national agricultural income. Livestock farming is predominantly extensive (Bengouga et al., 2019) and mainly distributed in the North part of the country. Tuberculosis (TB) is an ancient disease, discovered in the relics of ancient Egypt, India and China (Good et al., 2018). In 1882, Robert Koch presented his findings in his famous lecture at the Berliner Physiologischen Gesellschaft in Berlin, where he described the isolation of the causative agent from human and animal samples. In developing countries, TB represents a major cause of organ condemnation according to...
meat inspection of cattle carcasses at slaughterhouses, after fascioliosis, hydatidosis and cysticercosis. Therefore, TB is of great economic importance in these countries (Addis, 2017). In Algeria, the animal TB status is not clear, due to the lack of an official data, in addition, the scale of zoontic transmission is not well documented. Nevertheless, the cultural practices that exist could facilitate the elucidation of transmission between cattle and humans. Meat inspection in slaughterhouses play an important role to fight against bTB in Algeria. The aim of this study is to generate data on the prevalence of bTB in Algeria, through a risk factor study of abattoir records and thus to contribute to the knowledge on the epidemiology of the disease. This study can serve as a background information for guidelines for control measures.

MATERIALS AND METHODS

POSTMORTEM INSPECTION PROCEDURES

The TB detection was conducted by the abattoir assistant meat inspector, according to the guidelines recommended by the Algerian Ministry of Agriculture and Rural Development (MADRP). Inspection of carcasses for the presence of visible lesions involves palpation and incision into thin sections of lymph nodes (Ln). The cut surfaces were examined carefully for the presence of abscess, caseous mass and tubercles.

COLLECTION OF SAMPLES

The samples (n = 184) were collected from bovine carcasses (n = 3848) after meat inspection in four slaughterhouses, during the rainy season from October to March and the dry season from April to September. Samples of approximately 50g from each organ or Ln are randomly collected using a sterile single-use scalpel. These samples were placed in hermetically sealed, sterile, single-use vials and labeled with the age, the sex of animal and the collection date.

ZIEHL-NEELSEN ACID FAST STAINING AND CULTURE

Samples are transported to the Reference Laboratory of Mycobacteria and Control of Drug Resistance at Pasteur Institute of Algeria (IPA). From 184 positive carcasses a sample of 105 tissues lesions are examined by culture and direct smear microscopy using Ziehl-Neelsen staining for recording the presence of AFB. The tissue samples are decontaminated according to modified Petroff’s method (Tripathi et al., 2014). Briefly, an amount of 5ml of 4% NaOH solution was added to about 10g of tissue minced by a mortar in 4ml of sterile distilled water, and incubated for 15min. The suspension is neutralized by 10 ml of sterile distilled water and then centrifuged at 3000rpm for 20 min. The pellet was used as an inoculum to Löwenstein-Jensen as solid culture medium. The culture of mycobacteria was performed in a minimum of 8 to 12 weeks at

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### Table 1: Oligonucleotide primers used for molecular identification of mycobacteria isolates and sizes of the PCR products.

| Locus | Primer name     | Primer sequence                      | Present (M. tuberculosis) | Deleted (M. bovis) |
|-------|-----------------|--------------------------------------|---------------------------|-------------------|
| RD 9  | RD9_FlankF      | AACACGGTCACGTGTGTCGTA               | 396                       | 575               |
|       | RD9_FlankR      | CAAACCAGCAGCTGTGTCGTTG              |                           |                   |
|       | RD9_InternalR   | TTGCTTCCCCGGTGTGTCTG               |                           |                   |

### Table 2: Prevalence of bTB in four slaughterhouses from North Algeria

| Abattoir            | Cattle Examined (No.) | Positive (No.) | Positive (%) | Mean (%) | Prevalence (%) | IC (%) | p        |
|---------------------|-----------------------|----------------|--------------|----------|----------------|--------|----------|
| Hussein Dey         | 2207                  | 98             | 53.3         | 4.44     | 0.46-0.60      | <0.0001|
| El Harrach          | 1167                  | 62             | 33.7         | 5.31     | 0.26-0.40      |         |
| Hadjout             | 344                   | 17             | 9.2          | 4.94     | 0.05-0.13      |         |
| Dellys              | 130                   | 7              | 3.8          | 5.38     | 0.02-0.08      |         |
| Total               | 3848                  | 184            | 25           | 4.78     | 0.04-0.05      |         |

IC: Interval of confidence is of 95%

### Table 3: Prevalence of bTB with respect to sex

| Sex     | Positive (No.) | Prevalence (%) | IC (%) | p       |
|---------|----------------|----------------|--------|---------|
| Female  | 39             | 21.2           | 0.16-0.28|         |
| Male    | 145            | 78.8           | 0.72-0.84| <0.0001|
| Total   | 184            | 100            |        |         |

IC: Interval of confidence is of 95%

### Table 4: Prevalence of bTB with respect to age

| Age      | Positive (No.) | Prevalence (%) | IC (%) | p       |
|----------|----------------|----------------|--------|---------|
| < 2 years| 72             | 39.1           | 0.32-0.46| <0.0001|
| 2 to 6 years| 79        | 42.9           | 0.36-0.50|         |
| ≥6 years | 33             | 17.9           | 0.13-0.24|         |
| Total    | 184            | 100            |        |         |

IC: Interval of confidence is of 95%

### Table 5: Distribution of TB lesions in slaughtered cattle

| Localization            | Positive (No.) | Prevalence (%) | IC (%) | p       |
|-------------------------|----------------|----------------|--------|---------|
| Sternal Ln              | 1              | 0.5            | 0.001-0.03| <0.0001|
| RetrohepaticLn          | 7              | 3.8            | 0.02-0.08|         |
| Pulmonary (Inspector) Ln| 4              | 2.2            | 0.01-0.05|         |
| Mediastinal Ln          | 6              | 3.3            | 0.02-0.07|         |
| Prescapular Ln          | 3              | 1.6            | 0.01-0.05|         |
| Retropharyngial Ln      | 9              | 4.9            | 0.03-0.09|         |
| Tracheobronchial Ln     | 132            | 71.7           | 0.65-0.78|         |
| Pleura                  | 4              | 2.2            | 0.01-0.05|         |
| Kidney                  | 1              | 0.5            | 0.001-0.03|         |
| Digestif tract          | 1              | 0.5            | 0.001-0.03|         |
| Hepatic tissue          | 7              | 3.8            | 0.02-0.08|         |
| Lung tissue             | 9              | 4.9            | 0.03-0.09|         |
| Total                   | 184            | 100            |        |         |

IC: Interval of confidence is of 95%, Ln: Lymph node
### Table 6: Prevalence of bTB with respect to season

| Season | Positive (No.) | Prevalence (%) | IC (%) | p       |
|--------|----------------|----------------|--------|---------|
| Dry    | 140            | 76.09          | 0.7-0.82 | <0.0001 |
| Rainy  | 44             | 23.91          | 0.17-0.30 |         |
| Total  | 184            | 100            |        |         |

IC: Interval of confidence is of 95%

### Table 7: Prevalence of bTB in bovine carcasses analyzed by direct smear microscopy

| Microscopy | Prevalence (No.) | Prevalence (%) | IC (%) | p       |
|------------|------------------|----------------|--------|---------|
| Positive   | 60               | 57.1           | 0.48-0.66 | >0.05   |
| Negative   | 45               | 42.9           | 0.34-0.52 |         |
| Total      | 105              | 100            |        |         |

IC: Interval of confidence is of 95%

### Table 8: Prevalence of bTB in bovine carcasses analyzed by culture technique

| Culture     | Prevalence (No.) | Prevalence (%) | IC (%) | p       |
|-------------|------------------|----------------|--------|---------|
| Contaminated| 2                | 1.9            | 0.01-0.07 | <0.05   |
| Positive    | 60               | 57.1           | 0.48-0.66 |         |
| Negative    | 43               | 41.0           | 0.32-0.51 |         |
| Total       | 105              | 100            |        |         |

IC: Interval of confidence is of 95%

**Figure 1:** Bovine tuberculosis lesions discovered in slaughterhouses. Pleura with solid spherical formations covered with yellowish nodules (Pearl disease) (a), tracheobronchial Ln section with yellowish caseous consistence (b), digestive tract showing TB lesion in the rumen wall(c).

**DISCUSSION**

Of 3848 bovine carcasses inspected, 184 (4.78%) presented suspicious lesions of bTB (Table 2). This prevalence is higher than those reported in Algeria by Sahraoui et al. in 2008 (3.58%) and Ayad et al. in 2020 (2.06%), however, it

**Figure 2:** Acid-fast bacilli appeared as pinkish coccobacilli in a bluish background detected in lymph nodes from calve (x100).

**Figure 3:** Agarose gel electrophoresis of the amplified MTC product. A, B: DNA marker (80-1000pb), 1: negative control, 2: positive control *M. bovis* (BCG), 3: positive control *M. tuberculosis* (H37Rv), 4-8: MTC PCR product.
Patterns of Mekonnen et al. (2019), indicate that bTB re-
actors increase to a peak at 5 to 7 years of age. This increase
could be linked to a longer exposure time to the *M. bovis*.
Overall, the observation of lesion in calves could be ex-
plained by congenital transmission in utero. Pseudo-ver-
tical transmission (like ingestion of contaminated colos-
trums) is often referred to the close contact between cows and their calves.

Calves and bulls are destined earlier for slaughter in order
to cover the population’s demand for meat consumption,
while females are kept until an advanced age for repro-
ductive reasons and to exploit maximum milk production
during their lifetime. Brooks-Pollock et al. (2014) showed
that reactor rates to the comparative cervical test for bTB
increased with age for the first 2-3 years for beef, but also
for dairy cattle in Great Britain. In fact, this was expected
for cow-calf herds, since calves spend most of their time
with their dam before being weaned and moved away
(Rossi et al., 2019).

Most of bTB like lesions were anatomically located in the
thoracic cavity (84.8%) (Table 5, Figure 1a), mainly in res-
piratory tract. These were mainly in the tracheobronchial
Ln (71.7%) (Figure 1b), lung tissue (4.9%), mediastinal Ln
(3.3%), pleura (2.2%), pulmonary (Inspector) Ln (2.2%) and
sternal Ln (0.5%). Another localization was observed in the
liver tissue and associated (retrohepatic) Ln (7.6%) and
retropharyngeal Ln (4.9%). The TB lesions were less
noticed in the prescapular Ln (1.6%), in the digestive tract
(0.5%) (Figure 1c) and in the kidney (0.5%).

The route of transmission determines the location and
spectrum of lesions observed in bTB (Serrano et al., 2018).
According to Michel et al. (2010), overall, the TB lesions
most often occur in the lungs and the retropharyngeal,
bronchial, mediastinal and mesenteric Ln. Our results are
in agreement with those of Menzies and Neill (2000), in
which TB lesions are observed mostly in thoracic cavity
57%. Similarly, Boukary et al. (2012) identified 92.77% of
gross lesions in the lungs. Detection of lesions in the tho-
racic cavity (Ln) suggests that the respiratory tract is the
entry point for *M. bovis* infection.

On the other hand, ingestion of *M. bovis* from contaminat-
ed pasture, feed, or water usually results in mesenteric Ln
lesions (Menzies and Neill, 2000) and few or no visible les-
sions on the intestinal wall (Neill et al., 2001), which could
explain the presence of this lesion in our study at a very low
frequency (0.5%) (Figure 1c). The presence of lesions in the
liver could be related to transplacental transmission to the
fetus probably as a result of TB endometritis from the
cow (Domingo et al., 2014). Corner et al. (1990) observed
that the most frequently involved organ was the medi-
astral Ln (28.2%), tracheobronchial Ln (18.0%), lung tissue (8.0%),
mesenteric Ln (2.9%), parotid Ln (2.4%) and cervical cau-
dal Ln (2.4%). In summary, although the aerogenous route is
the most common pathway of infection in both domestic
ruminants and human beings and most cases of bTB in-
volve the lungs and respiratory Ln (O’Reilly and Daborn,
1995), the oropharyngeal route is also a frequent pathway of
entry that is probably more relevant than previously
thought (Domingo et al., 2014).

Analysis of data recorded in the present study shows that
bTB is present during all seasons, but with high frequency
during dry season (p<0.05) particularly July and August
(Table 6). According to Awah-Ndukum et al. (2010) from
Cameroon, the detection of TB lesions is not influenced by
season but were high during stressful periods such as inter-season and peak-season periods. Boukary et al. (2012), Raufu and Ameh (2010) and Sa’idu et al. (2017) from Nigeria reported that the frequency of lesions was higher at the beginning of the rainy season (July and August).

The high frequency of TB lesions during the dry season (76.09%), observed in our study, could be attributed to the massive destocking of animals by farmers during this season, and the high number of cattle presented for slaughter especially during summer which corresponds to the period of traditional celebrations and thus a high demand for meat. The period of winter concurs with the return of animals from transhumance. During the winter season, animals are confined to their farms because of the rains, thus, so close contact between animals promotes the transmission of TB infection. Animals are infected during the cold season and express the disease after a long incubation period during the dry season.

In current investigation, 60/105 (57.1%) samples tested were AFB positive by smear microscopy, 45 (42.9%) were negative (Table 7). The number of positive samples for AFB was greater than the negative ones with no significant difference (p > 0.05). This analysis demonstrates that Ziehl-Neelsen technique gives a good sensitivity for the detection of AFB in our samples.

Results of Aljameel et al. (2014) showed that out of 163 analyzed samples, 124 (76.1%) were smear positive while 39 (23.9%) were AFB negative. One of the explanation supplied could be related to the small number of samples examined in our study. Three modes of bacilli appearance are distinguished in the present study, most of them are single or grouped into two or three bacilli long filaments, resembling the M. tuberculosis species. Other forms, such as small shaped bacilli were observed, but rarely coccobacilli (Figure 2). The bacilli were usually pink colored. Nevertheless, conventional methods for TB detection using AFB microscopic staining are insensitive due to the potential confusion with other AFB genera such as Gordonia, Nocardia, Rhodococcus and Tsukamurella (Talip et al., 2013). From a total of 105 samples cultured, 60 were positives (57.1%), 43 were negative (41.0%) and 2 were (1.9%) contaminated (Table 8). The positive cultures (n=60) analyzed by PCR RD9, belonging to the MTC (Figure 3). Courcoul et al. (2015) study shows, that PCR is more sensitive and specific than bacteriology. This makes PCR a useful tool that could potentially become an official bTB diagnostic test in Algeria.

CONCLUSION

The post-mortem examination results revealed that the prevalence of bTB in Algeria is relatively low (4.78%). It is suggested that on the basis of this finding, the TB could not be underestimated, because in some cases lesions may not be sufficiently developed to be able to detect during routine inspection at the slaughterhouses. Gender, age and season were recognized as important risk factors associated with bTB infection. Males (78.8%) were found more susceptible to infection than females (21.2%); young (39.1%) and adult (42.9%) animals were more susceptible to infection than older one (17.9%); and dry season (76.09%) seems to be the most favorable period for the spread of the disease as compared to rainy season (23.91%). These results should interpolate the competent health authorities to adopt effective control strategies and appropriate measures for the prevention of bTB in Algeria, and development of laboratories specialized in the diagnosis of animal TB as well.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS CONTRIBUTION

Conceptualization: FB, MN, TMH
Data curation: FB
Formal analysis: SZ, FB
Investigation: FB
Methodology: FB
Supervision: TMH, MN
Visualization: IM, FB
Writing – original draft: FB
FB conceived, designed the study and drafted the manuscript under the supervision of TMH and MN. FB designed the experiment protocol. FB collected and analyzed samples. FB and SZ performed the statistical analysis. FB revised the manuscript under the supervision of IM and TMH. All authors read and approved the final manuscript.

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