

*Full Length Research Paper*

# Investigation about the bovine tuberculosis in two Algerian slaughterhouses

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**In Algeria, real situation of bovine tuberculosis is unknown. It is widely suspected at slaughterhouses but must be supplemented by laboratory tests. In this study, we report the results of a survey conducted during the period from August to November 2007. From a total of 7250 carcasses examined, 260 showed suspicious lesions of tuberculosis, representing a prevalence of 3.58%. The distribution of injuries shows frequently a violation of respiratory lymph nodes with a rate of 76.92%. Microscopic examination of 260 pieces analyzed showed positive samples in 28.85% of the cases. The isolation and identification of isolates have confirmed 134 positive cultures, at a rate of 51.54%. Among these, 86.57% are represented by *Mycobacterium bovis* stains and 13.43% by a non tuberculosis mycobacteria strain. Hence, although the eradication programme has been operational for several years, this disease still remains widely apparent among Algerian territories.**

**Key words:** Bovine tuberculosis, slaughterhouse, microscopy, culture, identification.

## INTRODUCTION

Bovine tuberculosis remains one of the most widespread and devastating disease in cattle herds, particularly in developing countries, like in Africa (Delafosse, 1995; Benkirane, 1998; Bonsu, 2000). Its economic impact (big losses by seizure at slaughterhouses and reduced milk production) and its importance in public health have led to the development of control and eradication programs in many countries.

For the others countries of the Maghreb, like Morocco, Fikri (1999) reported that the rate of infection was 1.8%, detected by tuberculin- positive test and in Tunisia, An annual screening and culling is established for decades and the result is a significant decrease in cases of bovine tuberculosis (OIE, 2001).

In Algeria, in addition to a multi-year sanitation program in cattle initiated since 1995 and in spite of its position as a mandatory reporting disease (Executive Decree No.

95\_66 of 22/02/1995), recent OIE reports shows that the recorded rate of infection was respectively of 0.49 and 0.39% in 2001 and 2003 (OIE, 2004).

The bovine tuberculosis is a highly infectious disease caused by called *Mycobacterium bovis* bacteria. This disease can affect almost all mammals including humans. This bacterium usually infects lymph nodes and then spreads to the other organs like “lungs”. TB develops slowly in affected animals. General symptoms include weakness, loss of appetite and weight and a variable fever. When lungs are highly affected, we can observe on intermittent fifths of cough.

Nowadays, tuberculosis detection is often done at slaughterhouses. The discovery of characteristic granulomatous lesions in the lymph nodes or organs when inspecting animal carcasses sign the presence of the disease (Artois et al., 2000).

In this study, we intend to make a survey by seeking the causative agent of the disease and by identification of specimens from two slaughterhouses from the north of Algeria.

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**Table 1.** Specimens collected at slaughterhouses.

Organ/ tissue	Number	Percentage
Lymph nodes	200	76,92
Lungs	41	15,77
Pleura	4	1,54
Liver	8	3,08
Mesentery	2	0,77
Head	5	1,92
Total	260	100

**Table 2.** Results of the microscopic samples.

AFB- smear	Number of samples	Percentage
positive	75	28,85
negative	185	71,15
total	260	100

## MATERIALS AND METHODS

### Animals

This study was conducted in two slaughterhouses (Algiers and Blida) situated in the north of Algeria during the period from August to November 2007. A total of 7250 animal were slaughtered, we highlighted suspicious lesions of tuberculosis from 260 carcasses.

### Samples

Samples were collected from observed lesions, and deposited into sterile universal bottles with a fact sheet. All samples were transported in ice to the Algerian pasteur institute laboratory.

### Treatment of samples

In laboratory, specimens were cut into small pieces using sterile blades and petri dishes. Fragments were first crushed and homogenized with a mortar and pestle. These fragments were submitted to a microscopic examination and bacterial culture.

### Microscopic examination

Direct smears were prepared from tissues presenting tuberculosis lesions and stained by using Ziehl Neelsen acid –fast staining technics. This method consists of using a rigid cove platinum, first burn and cooled. The collected sample is spread in a thin layer in the center of the blade. The smears were stained with carbol-fuchsin (three rapid passages on the flame), and then washed with tap water and decolourised with sulphuric acid and alcohol. Every slide is washed under tap water between each step, and then counter- stained with methylene blue for 1 min and read in an optical microscope (X 100 and oil immersion objective).

### Decontamination and inoculate

Homogenates were decontaminated by adding 4 ml of 4% sulphuric acid, and then neutralized by 6% NaOH using the blue

bromothymol as an indicator. The neutralization was achieved by crossing the yellow color to green, and then centrifuged at 3000 g for 15 min.

The sediment was inoculated onto a set of Lowenstein- Jensen slants supplemented with 0.4% sodium pyruvate (L-J pyruvate) and glycerol (standard L-J). Cultures were incubated at 37°C for up to 12 weeks, with weekly observation for discernible growth. Growth considered as mycobacterium was examined using the Ziehl Neelsen technic for confirmation of acid fast bacilli (AFB).

### Identification

Initial identification of mycobacterium species was based on the rate of growth and colony morphology. The identification consisted of achieving three biochemical conventional tests (De Kantor et al., 1998; Vestal, 1978), namely, the nitrate reduction, the niacin production and catalase thermolabile activity to 68°C (Kent and Kubica, 1985).

These tests were conducted for the identification of mycobacterium and to differentiate strains of *Mycobacterium tuberculosis* complex strains from mycobacterium non tuberculosis.

## RESULTS

The inspection of 7250 carcasses revealed that 260 between them were carrying suspicious lesions of tuberculosis.

The overall prevalence of suspect lesions was of 3.58%. These lesions were mainly found in the lungs and their lymph nodes and in the liver. The origin of injuries based on samples is reported in Table 1.

Our results report that over of 76% of lesions were located in the thoracic lymph nodes, 15.77% in the lungs and the remaining 8.23% were located in the pleura, liver, mesentery and head.

### Direct microscopy

From the 260 smear made for microscopic examination, only 75 were found positive. These results are reported in Table 2.

The proportion of positive specimen's smears was lower than negative smears (28.85%) vs. (71.15%).

### Bacterial culture

Results of the bacterial culture after 3 months of incubation are reported in Table 3.

In total, we have isolated 134 positive culture (51.54%), whereas the remained 106 cultures were negative (40.77%); only 7.69% of the cultures were contaminated.

### Culture morphology and biochemical identification

Results of the biochemical identification are reported in Table 4.

**Table 3.** Results of bacterial cultures.

Culture	Number	Percentage
Positive	134	51.54
Negative	106	40.77
Contaminated	20	7.69
Total	260	100

**Table 4.** Results of the biochemical identification.

Culture	Number	Percentage
<i>M. bovis</i>	116	86,57
NTM	18	13.43
Total	134	100

The cultures and biochemical tests have identified 86.57% strains of *M. bovis* and 13.43% strains of Non-Tuberculosis Mycobacterium (NTM).

## DISCUSSION

In Algeria, the real situation of bovine tuberculosis remains unknown. Only the screening positive cases are reported in the official statistics of the Ministry of Agriculture. The OIE reported 434 cases of bovine tuberculosis detected by tuberculin- positive test for the year 2003. No epidemiological data has so far been published. Hence, the true status of the cattle population in respect of tuberculosis remains unknown and these results will not be a comparison with other studies.

Distribution of injuries shows that 76.92% of them concern mainly lymph nodes of the thoracic cavity. A similar observation was made by Fikri (1999) and Teklu et al. (2004) which report that 74 and 84% of lesions respectively are found in the chest and lungs lymph nodes, the prevalence of TB lesions in the airways is explained by the transmission of the disease through inhalation, which is considered the main route of transmission (O'Reilly and Daborn, 1995).

The direct microscopy revealed a low percentage of AFB of 28.85% in slaughterhouses. These results are not surprising, since the microscopic examination is not sensitive and do not become positive if the sample contains less than  $10^4$  / ml (Carbonnelle et al., 2003).

Moreover, direct microscopy is regarded as the least sensitive test for detecting AFB in relation to all methods of diagnosis (Baron et al., 1994; Cernoch et al., 1994). Despite the low-known sensitivity to this method of diagnosis, our results are comparable to those obtained in other studies conducted on samples from slaughterhouses (21%) (Diguimbaye, 2006).

For the bacterial culture: we got 51.54% positive ones, 40.77% of negative ones, and only 7.69% contaminated at the slaughterhouses.

Results of culture are also insufficient, as long as the percentage of positive cultures east of 51.54%: lack of crop growth in some specimens with injuries at the time of inspection can indicate misclassification of non-tuberculosis lesions but might also possibly be due to the absence of viable mycobacterium in calcified tuberculosis lesions. In completely calcified lesions, tubercle bacilli are dead and therefore no growth will be obtained upon culture (Gracy, 1986; Quinn et al., 1994; Teklu et al., 2004). Our results are low compared to the results reported by Diguimbaye (2006) which on 65.7% positive cultures, 27.3% negative cultures and 7% contaminated crops.

For culture morphology and biochemical tests, our results show 86.57% of *M. bovis* crops and 13.43% cultures of non tuberculosis mycobacterium at slaughterhouses. Our results are different from those reported by Diguimbaye (2006), which indicate 41.66% of *M. bovis* and 58.33% non – tuberculosis mycobacterium.

So, these results show that cattle in Algeria are more affected by tuberculosis than by other mycobactéria.

## Conclusion

This study has helped to determine the prevalence at slaughterhouses and identify mycobacterium responsible for tuberculosis in Algeria.

Bacteriological test has a great interest for both human and animal health, and remains the best method for diagnosing tuberculosis.

These elements contribute to a better understanding of the situation of bovine tuberculosis in Algeria.

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