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Full Length Research Paper

Survey on tuberculosis goats in two slaughterhouses in Algeria

Naima Sahraoui, Samir Zelleg, Nadir Yousfi , Jakob Zinsstag and Djamel Guetarni*

Université Saad Dahlab, Blida, Algeria.

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In Algeria, the absence of means of screening for tuberculosis goats on the living animal, the post-mortem inspection of goats' carcasses at slaughter is the only way possible, but this is not always the case because of the illegal felling of this species. This study was conducted in two slaughterhouses in Algeria, over a period of two months in order to determine the proportion of goats' tuberculosis and highlight the agents. Out of 995 carcasses inspected, 60 showed suspicious lesions of TB, a proportion of 6.03%. Microscopic examination showed a positivity of 13.33%. As for culture, a percentage of 10.01% of positive cases was noted. The molecular diagnostic tools have revealed no strain of *Mycobacteium tuberculosis* complex. While the sequencing of gene16SrRNA showed two strains belonging to *Corynebacterium xerosis*, a strain of non tuberculous mycobacteria, a strain of *Corynebacterium sp.* whose species has not been determined, a strain of *Corynebacterium pseudotuberculosis* and a strain of *Desulfosporosinus sp.* Therefore, we confirmed the absence of goats' tuberculosis in both slaughterhouses.

Key words: Goats' tuberculosis, Inspection, Algeria, slaughterhouses, sputum, culture, sequencing.

INTRODUCTION

The goats' livestock estimated at 2.5 million head is more concentrated in the steppe, mountainous region and oasis. This livestock is represented by the Arabic goat which includes two types, the M'Zab goat and the Kabyle goat, the latter is from this region (INRA, 2003).

In recent years, several zoonotic diseases begin to spread in the goats' livestock as the tuberculosis whose responsible agent is the *Mycobacterium caprae* which will be probably put in the category of germs with a risk level of 3. This disease causes heavy economic and public health losses (INSP, 2007).

In Algeria, the problem with goats' TB is neglected. There is virtually no reliable data on the extent of the disease. The information on the prevalence of goats' TB is lacking. Furthermore, we wish to mention that the goats' livestock is not subjected to any TB control test. A small number of goats is slaughtered and subjected to carcasses inspection at slaughterhouses, while monitoring of slaughterhouses allows only minimal disposal of infected carcasses in slaughter plants because many are made clandestinely.

To better understand the situation of goats' TB, it is necessary to conduct a bacteriological diagnosis which is essential in the management of the disease, since it can identify the bacteria. However, because of the similarity of cropping and biochemical characteristics of mycobacteria (Aranaz et al., 2003), new diagnostic tools based on molecular biology have been developed to accelerate the detection and identification of these agents in biological samples (Zintilini, 2003).

MATERIALS AND METHODS

Animals

This study was conducted in two slaughterhouses (Tazmalt and Souk El Tenine) located at 85 and 35 km, respectively, from the

*Corresponding author. E-mail: dguetarni@gmail.com.

Table 1. Results of microscopic examination for samples of suspected goat's TB in Béjaia abattoir (July-August 2008).

Microscopy	Number of specimens (n)	Percentage (%)
Positive	8	13.33
Negative	52	86.66
Total	60	100

wilaya of Bejaia, over a period of two months (July and August of 2008). During this period, a total of 995 goat carcasses were inspected.

Samples

Sixty (60) samples showing suspicious lesions of TB were collected. The latter were achieved mainly in the lungs and their major glands (tracheobronchial and mediastinal) and transported under ice (+4°C) to the service of tuberculosis and my cobacteria of the Pasteur Institute of Algeria.

Treatment of samples

In the laboratory, we proceeded to the drying of samples, using Petri dishes, and disposable blades. Using sterile mortar, fragments of the samples were finely crushed with a pestle. The crushing product, thus obtained is decontaminated with sodium hydroxide by the method of Petroff (1915).

Microscopic examination

This technique is based on the fundamental nature of mycobacteria, which is acid-fast resistance, allowing the identification of acid-fast bacilli-resistant (AFB) by microscopy (Amer, 1915).

Using a loop, we take a fragment of pus. The content of the loop is spread in thin layer in the center of the blade. The coloring is done with heat every three minutes by adding fuchsin, so that the blade is always covered. Thus, we proceed to the discoloration with sulfuric acid. The blade is covered again with alcohol at 90° for 05 min. Finally, the last time is to recolor for 30 s the blade by the solution of methylene blue, followed by washing and drying. The blade is observed with a microscope.

Bacterial culture

The final pellet was planted on four tubes of Lowenstein-Jensen, two fortified with pyruvate and two with glycerol. These tubes are then placed in an oven at 37°C for 12 weeks. A week ly monitoring of the growth of colonies was done.

Identification

The identification of strains is based on the time of appearance of colonies and their morphology.

Extraction of deoxyribonucleic acid (DNA)

Positive cultures were transported to the National Center for Mycobacteria in Zurich (Switzerland) for DNA extraction, following the protocol described in the kit (InstaGeneTM Matrix, Bio-Rad).

Molecular characterization

Spoligotyping

The technique of "typing by oligonucleotide spacers DR locus" is used for the identification and typing of strains belonging to the *Mycobacterium tuberculosis* complex, is made from six different strains in the Swiss Tropical Institute in Switzerland according to the protocol published by Kamerbeek et al. (1997).

Sequencing

Sequencing was performed at the National Center for Mycobacteria in Zurich, as described by Zucol et al. (2006) to identify these bacterial species. Species identification was performed by comparison with sequences of SmartGene Integrated Database Network System (IDNSTM) 3.4.0. The criteria for species identification are required as reported by Bosshard et al. (2003).

RESULTS

In the present study, 995 goat carcasses were inspected and 60 had suspicious lesions of TB, thus, the proportion of suspicious lesions of goats TB obtained in the two slaughterhouses in the wilaya of Bejaia is 6.03%.

Laboratory diagnosis

Microscopic examination

The results of microscopic examination, reported in the Table 1, showed that in a total of 60 samples, eight were smear positive, 13.33%.

Bacterial culture

The results of bacterial culture, reported in the Table 2, showed that the percentage of positive cultures was 10.01%.

Table 2. Results of bacterial culture for samples of suspected goat's TB in Béjaia abattoir (July-August 2008).

Culture	Number of bacterial culture(n)	Percentage (%)
Positive	06	10.01
Negative	49	81.66
Contaminated	05	08.33
Total	60	100

Table 3. Identification of other bacterial species by sequencing the gene 16 S rRNA.

16 S rRNA Identification	Number	Percentage (%)
M. terrae complex	1	16.66
Corynebacterium sp.	1	16.66
Corynebacterium xerosis	2	33.33
Corynebacterium pseudotuberculosis	1	16.66
Desulfosporosinus sp.	1	16.66
Total	6	100

Spoligotyping

The results of spoligotyping revealed the absence of Mycobacteria strains belonging to the *Mycobacterium tuberculosis* complex.

Sequencing

The results of sequencing of 16SrRNA gene of the 6 isolates reported in the Table 3 show that:

1. Two strains belonging to *Corynebacterium xerosis* (99% homology).
2. A strain of non-tuberculous mycobacteria (NTM) 99.5% homology with *M terrae*.
3. A strain showed a similarity to *Corynebacterium sp*. (98% homology) whose species has not been determined.
4. A strain showed a similarity to *Corynebacterium pseudotuberculosis* (99% homology);
5. A strain *Desulfosporosinus* sp. (96% homology).

DISCUSSION

On a set of 995 inspected goat carcasses at two abattoirs in the wilaya of Bejaia, 60 showed lesions suspicious of goat TB, a proportion of 6.03%. The percentage initially high, do not read the actual proportion of TB in those two slaughterhouses, and this is due to lack of specific concepts characterizing the TB lesions in this species, moreover, the involved veterinary services have not registered any case, neither this year nor previous years.

We reported for the first time, that the *M. caprae* was isolated and characterized in Algeria in the bovine. These results imply the transmission of TB between the two species (cattle and goats) as a result of their cohabitation (Sahraoui et al., 2009).

TB is often confused with the three diseases common in goats, as reported in the work of Thorel (2003), namely: bronchopneumonia and hepatitis, strongly parasitic which is also characterized by eosinophilic adenitis more pronounced, and finally, the caseous disease (pseudo-tuberculosis) lymphatic location, lung or liver. All suspicious lesions were treated by two bacteriological examinations, namely the direct examination and bacterial culture.

Direct examination of smears revealed 13.33% of positive slides; this reflects the difficulty of differential diagnosis between goats' TB and the three diseases already mentioned. Furthermore, it should be noted that, microscopic examination is not specific because all mycobacteria are acid-fast resistant or sensitive, since it is only positive when the sample contains 10^4/ml AFB (Carbonnelle et al., 2003).

The bacterial culture examination revealed 10.01% for positive cultures. This low percentage compared to the direct examination is due to the insufficient sampling in bacilli and AFB youth damage, whose face is immature as a result of manipulation (decontamination, centrifugation and agitation). The results of the spoligotyping technique, revealed the absence of mycobacteria from *Mycobacterium tuberculosis* complex. The sequencing of the gene 16SR RNA is a very useful tool for the identification of unusual clinical isolates or

those that can not be easily identified by conventional phenotypic methods (Vela, 2003; Drancourt and Raoult, 2005; Gibello et al., 2005). Sequencing of 16S r RNA gene of six strains tested negative, the technique of spoligotyping was used to identify two strains of *Corynebacterium xerosis* and a third, whose species has not been determined, a strain of *Mycobacterium Terrae* which shows a similarity to *Corynebacterium pseudotuberculosis* and strains of *Desulfosporosinus* sp.

In this study, *Corynebacterium xerosis* was the most commonly isolated bacterium (2/6). This study describes the first identification of *Corynebacterium xerosis* in Algeria from animal clinical specimens, which was confirmed by sequencing the 16S rRNA gene. Studies on Corynebacterium and Mycobacterium indicate that these two species belong to the same group. The latter contains a number of pathogenic bacteria that includes the genus Rhodococcus, Nocardia, Corynebacterium and Mycobacterium (Goodfellow and Alderson, 1977).

In this study, only one isolate out of six had an atypical mycobacterium whose species is close to *M. terrae*. Indeed in Africa, works on the non tuberculosis mycobacteria infections in animals are rare. The study of Churchyard et al. (1999) and Corbett et al. (1999) conducted in South Africa indicated that, the main strains responsible for mycobacterial disease are *M. kansasii* and *M. scrofulaceum*. Hamid et al. (2002) was also described as farcinogenes officer stuffed in some African countries. We were also able to isolate for the first time a strain of *Corynebacterium pseudotuberculosis*, which is responsible, in sheep and goats, for an infection known as caseous lymphadenitis.

This condition has been described in all countries where sheep farming is important (Pepin et al., 1999). It is characterized by the formation of pyogranulomes localized mainly in superficial lymph nodes (lymph nodes parotid, mandibular, retropharyngeal, precapsulaire, prefemoral, popliteal, retromammary) in the deep lymph nodes and lungs (Pepin et al., 1999). As such, this infection gives exclusively lymphadenitis and abscesses with granulomatous lesions, necrotizing and suppurative, must be regarded as an occupational zoonosis (Hemond et al., 2006).

Finally, a strain of *Desulfosporosinus* was also isolated. This type was first proposed in 1997 (Stackebrandt et al., 1997) to classify species previously known as orientis *Desulfotomaculum* (Spring and Rosenzweig, 2006). Currently, five different strains of *Desulfitobacterium* species are validly published (Vela et al., 2003).

Conclusion

Goat's TB can be regarded as absent at the two slaughterhouses. Nevertheless, the identification of microorganisms emerging and re-emerging seems to take precedence in goat farming in Algeria.

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