Bacilloscopy, Bacterial culture, and Spoligotyping of *Mycobacterium bovis* strains isolated from cattle in North Central Algeria

*Choâyb Mecherouk, Malika Ifticene and Nora Mimoune**

Abstract

This study aimed to assess bovine tuberculosis-like cases in three slaughterhouses in North Central Algeria and to confirm these suspected cases using microscopic, bacteriological examination, and molecular biology technique. We highlighted the factors influencing the prevalence of the disease. Also, the genomic profiles of *Mycobacterium tuberculosis complex* (MTBC) strains isolated by Oligonucleotide typing technique (Spoligotyping) were determined. At the abattoir level, bovine carcasses were routinely inspected to detect visible abnormalities including suspicious lesions of bovine tuberculosis (BTB). At the laboratory level (Pasteur Institute, Algiers), Ziehl-Neelsen staining, bacterial culture, biochemical study (nitrate reduction test), and spoligotyping were performed to confirm suspected cases. On a total of 1300 bovine carcasses, 100 presented BTB-like cases (7.69%). Animals over 5 years of age were more affected compared to other age groups. Lesions were observed more often in females than in males and cattle of local breed were the most exposed to BTB-like cases ($P<0.05$). Bacilloscopy data were positive for 44 of the 100 suspected samples (44%) while bacteriology showed that 56 cultures were positive, while 35 were negative and 9 were contaminated. Molecular spoligotyping of 40 *Mycobacterium* strains samples showed 19 spoligotype profiles of *M. bovis*, of which 50% of profiles have been previously detected in the Mediterranean area and the three spoligotype patterns not previously reported were named SB2651, SB2652, SB2653 (by http://www.Mbovis.org). Measures and means to prevent TB transmission among animals and to humans should be recommended, and more intensive investigations are required using both routine and molecular diagnostic techniques to understand and further explore *MTBC*.

Key words: *bovine tuberculosis; bacilloscopy; culture; spoligotyping; prevalence; factors*

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Introduction

Tuberculosis (TB) is a major cause of morbidity and mortality globally, affecting a variety of species (Silva et al., 2018). The causative agents of tuberculosis in mammals are homogenetic acid-fast bacteria referred to as the *Mycobacterium tuberculosis* complex (*MTBC*) (Costa et al., 2013). The primary pathogens associated with domesticated and wild animals include *M. bovis*, *M. caprae*, *M. microti*, *M. orygis* and *M. pinnipedii* (OIE, 2022). Some of these species such as *M. bovis* and *M. orygis* can occasionally cause zoonotic TB (Rossi et al., 2015; Otchere et al., 2019).

Globally, the actual number of zoonotic TB remains unknown, estimating 147,000 cases and 12,500 deaths in 2016 (Falzon et al., 2017; Gompo et al., 2020). Zoonotic transmission primarily occurs through close contact with infected cattle, consumption of contaminated products, or unpasteurized milk and milk products (Ayele et al., 2004). Therefore, the burden of zoonotic TB reflects the prevalence and distribution in cattle, as important reservoir, and further highlights cattle to human transmission through contaminated cattle products (El-Sayed et al., 2016).

Mycobacteria genotyping methods have proven to be important tools in understanding TB transmission and the epidemiological link (Ei et al., 2016). Spoligotyping is a useful molecular epidemiological typing method for investigating the circulation of *MTBC* strains and their evolutionary lineage, monitoring the dynamics of TB epidemics (Goguet de la Salmoniere et al., 1997; Gori et al., 2015). This method classifies strains based on the polymorphism of the unique spacer sequence between *M. tuberculosis* direct repeat regions. Although spoligotyping analysis has less discriminatory power than other molecular typing tools, such as pulsed-field gel electrophoresis, mycobacteria interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR) typing, and *IS6110* restriction fragment length polymorphism (RFLP) analysis, its PCR basis means that it can be tested reproducibly, simply, and quickly (Kamerbeek et al., 1997). Too high a discriminatory power might fail to detect clusters. Therefore, the slightly lower discriminatory power of spoligotyping analysis can be more advantageous for investigating overall circulating cluster trends. Spoligotyping is optimal for the overall investigation and quantification of TB because it is able to perform comparisons with numerical characters (Sauders et al., 2003).

This study was aimed at assessing the percentage of bovine tuberculosis-like cases in three slaughterhouses in the north central region of Algeria, and to confirm these suspected cases using microscopic and bacteriological examination, and molecular biology techniques. We highlighted factors associated with BTB. Finally, the genomic profiles of *MTBC* strains isolated by the Oligonucleotide typing technique (Spoligotyping) were determined.

Materials and methods

This study was performed over a period of 1 year, organized in two phases: the first consisting of sample collection at three slaughterhouses, and the second phase of analysis at the Tuberculosis Laboratory of the Pasteur Institute in Algiers (isolation, biochemical and molecular identification of samples).

Sample collection

Ante-mortem inspection

At three slaughterhouses located in the north central Algeria (Boufarik, El-Harrach, and Eucalyptus), 1300 cattle were inspected. These cattle came from

approved or unapproved farms, and therefore were subject to tuberculosis control or were not. Prior to examination of the animals, information was recorded on sex, breed (local, cross, imported), and age (under 2 years old, between 2 and 5 years old, and over 5 years old). This examination was done in order to avoid the slaughter of pregnant females and to carry out a stamping-out policy for animals afflicted by tuberculosis, brucellosis or enzootic bovine leucosis.

Post-mortem inspection

After bleeding, skinning and evisceration, routine inspection of the carcasses and offal was performed to detect visible abnormalities including suspicious lesions of bovine tuberculosis (BTB), as described previously (Gracey et al., 1999). Especially for BTB, the inspection consists of performing a systematic examination of all organs and incision with a clean knife of all lymph nodes (LN) as well as drained organs, as follows: Head: the mandibular and retropharyngeal LN; Lungs, trachea, tracheobronchial LN (cranial, right and left) and caudal mediastinal; Intestinal tract and the gastric and mesenteric LN; Genital tract. In detail, necropsy procedures were based on gross detection of typical tubercles that are whitish or yellowish in colour, yellowish granulomatous caseated lesions or sometimes 'gritty' calcification in the mentioned tissues (Ayad et al., 2020). Subsequently, while wearing a pair of sterile gloves, samples were taken from all suspected organs and their lymph nodes in sterile boxes transported by a cooler (+4°C) to the laboratory.

Sample analysis

Samples were taken from suspected lesions of BTB. These lesions were located on various organs, mainly in the lungs and their main LN (tracheobronchial and mediastinal) and in the liver. The sample was dissected using petri dishes and single-use scalpel blades. Using sterile mortars, sample fragments were finely ground using a pestle, occasionally with the use of sterile sand when the fragments were difficult to grind. The resulting grinding product was used for microscopic examination as well as culture.

Microscopic examination

Ziehl-Neelsen staining

The confirmatory histopathological diagnosis was done using Ziehl-Neelsen staining method for detecting acid-fast bacilli (AFB).

Bacterial culture

On a petri dish with a scalpel, very fine cuts were made on pieces of the biopsy sample. To decontaminate samples, the Petroff method modified with 4% NaOH was used. The suspension was stirred for 15 minutes, centrifuged at 3,000 rpm for 15 min, discarding the supernatants and adding H_2O for rinsing. We then proceeded to recentrifugation at 3,000 rpm for 15 min. From the sediment, Löwenstein Jensen (LJ) medium was inoculated and the smear was made. Then the bottles were incubated at 37°C until the multiplication of mycobacteria could be observed and then stored at room temperature.

Biochemical identification

Once the culture was declared positive, mycobacteria were identified using the nitrate reduction test.

Molecular identification by Spoligotyping

Molecular typing currently represents a major contribution to classical epide-

miological investigations concerning tuberculosis. The standardised method used in this study was that described by Kamerbeek et al. (1997), which is based on the detection of the polymorphism of the DR (Direct Repeat) region, known as the spoligotyping method. We chose it for two reasons: on the one hand it allows for the identification of the species of *MTBC* and differentiation of strains within the same species. On the other hand, it is a rapid method that can examine 43 strains in a single manipulation, in a period of only 48 hours. The results can be analysed by software or Excel and compared to a global database available online. The kit required for this technique is marketed by the firm Isogene® (Biosciences BV, Netherlands), consisting of a pair of primers from two reference strains for the internal control of a membrane. The advantage of this kit is that the membrane can be reused several times.

Statistical analysis

Statistical analysis was performed using the STATISTICA software (Version 10, Stat Soft France, 2003). Chi-squared test were used to analyse the factors associated with BTB-like cases. The results were considered significant when $P<0.05$.

Results

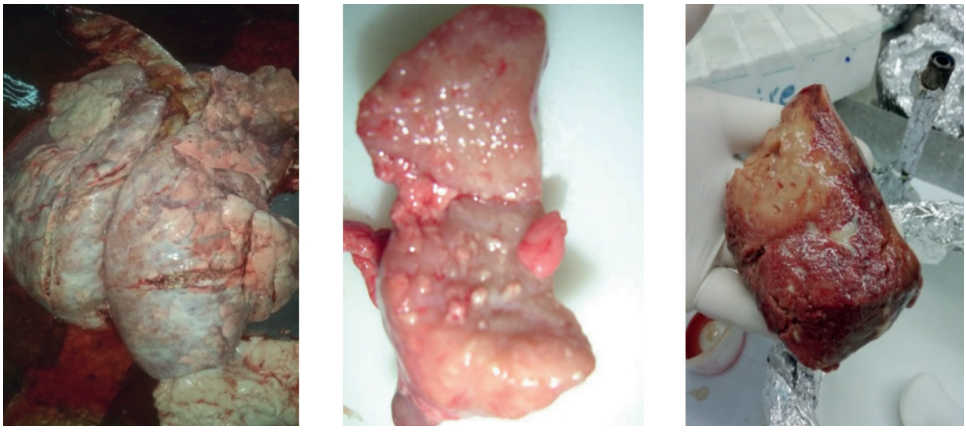
Bovine tuberculosis-like cases in abattoirs

The inspection of 1300 bovine carcasses at the slaughterhouses showed that 100 carcasses (7.69%) presented suspicious lesions of bovine tuberculosis (BTB) (Figure 1).

Factors influencing the occurrence of BTB-like cases

According to the data, the percentage of lesions was higher in females than in males ($P<0.05$). There is also a very significant difference in the proportions of BTB-like cases between age groups; animals over 5 years old were the most affected ($P<0.001$).

The study indicates a significant difference in the occurrence of BTB-like cases between the three breeds, so they did not show the same susceptibility to the disease and the local breed appeared



Lung TB | Lymph node TB | Hepatic TB

Figure 1. TB-like lesions detected at the slaughterhouse

Table 1. The occurrence repartition of BTB-like cases in relation to sex, age, breed, and location.

Factors associated with BTB-like cases		%
Sex	Male	43
	Female	57
	P	<0.05
Age	Less than 2 years	4
	2-5 years	35
	More than 5 years	61
	P	<0.001
Breed	Local	71
	Imported	6
	Cross	23
	P	<0.001
Localisation/Type	Organ	57
	Generalised	19
	LN	24
	P	<0.001

more susceptible than imported and cross breeds (P<0.001), (Table 1).

The data showed that lesions were more located in organs (57%) (mainly the respiratory system followed by the liver) and in LN (24%). Generalised BTB-like cases were noted in 19% (P<0.001).

Laboratory diagnosis

Results of direct examination (Bacilloscopy)

The results of microscopic examination are presented in figure 2.

According to Figure 2, bacilloscopy was positive for 44 of the total of 100 suspected samples (44%).

Results of bacterial culture

Bacteria culture revealed the following results (in comparison with the bacilloscopy data) (Figure 3).

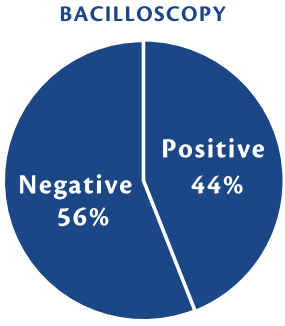


Figure 2. Bacilloscopy results

Of the total 100 cultures, 56 cultures were positive, 35 were negative, and 9 cultures were contaminated.

Biochemical identification

Testing the fresh cultures for nitratase was negative, which is characteristic of *M. bovis*.

	Spolygotype	Location of the Direct Repeat and the spacers of each spolygotype	Country
Bv 1	SB0856	■■□■■■■■□■■■■■□□■■■■■■■■■■■■■■■■■■■■■□□□□□	FRA
Bv 3	SB0856	■■□■■■■■□■■■■■□□■■■■■■■■■■■■■■■■■■■■■□□□□□	FRA
Bv 4	SB0856	■■□■■■■■□■■■■■□□■■■■■■■■■■■■■■■■■■■■■□□□□□	FRA
Bv 5	SB0856	■■□■■■■■□■■■■■□□■■■■■■■■■■■■■■■■■■■■■□□□□□	FRA
Bv 8	SB0856	■■□■■■■■□■■■■■□□■■■■■■■■■■■■■■■■■■■■■□□□□□	FRA
Bv 9	SB1204	■■□■■■■■□■■■■■□□■■■■■■■■■■■■■■■■■□■■■■■□□□□□	FRA
Bv10	SB1204	■■□■■■■■□■■■■■□□■■■■■■■■■■■■■■■■■□■■■■■□□□□□	FRA
Bv11	SB1204	■■□■■■■■□■■■■■□□■■■■■■■■■■■■■■■■■□■■■■■□□□□□	FRA
Bv12	SB1204	■■□■■■■■□■■■■■□□■■■■■■■■■■■■■■■■■□■■■■■□□□□□	FRA
Bv13	SB1204	■■□■■■■■□■■■■■□□■■■■■■■■■■■■■■■■■□■■■■■□□□□□	FRA
Bv14	SB0818	■■□■■■■■□■■■■■■□■■■■■■■■■■■■■■■■■□■■■■■□□□□□	FRA
Bv15	SB0119	■■□■■■■■□■■■■■□□■■■■□■■■■■■■■■■■■■■■■■□□□□□	BLG
Bv16	SB0119	■■□■■■■■□■■■■■□□■■■■□■■■■■■■■■■■■■■■■■□□□□□	BLG
Bv18	SB2257	■■□■■■■■□■■■■■□■■■■■■■■■■■■■■■■■■■■■□□□□□	MEX
Bv20	SB2257	■■□■■■■■□■■■■■□■■■■■■■■■■■■■■■■■■■■■□□□□□	MEX
Bv21	SB1209	■■□■■■■□□■■■■■□□■■■■■■■■■■■■■■■■■■■■■□□□□□	ITA
Bv22	SB1209	■■□■■■■□□■■■■■□□■■■■■■■■■■■■■■■■■■■■■□□□□□	ITA
Bv23	SB1640	■■□■■■■■□■■■■■□□■■■■■■■■■■■■■■■■■□■■■■□□□□□	SPA
Bv28	SB0120	■■□■■■■■□■■■■■□■■■■■■■■■■■■■■■■■■■■■□□□□□	UC ?
Bv29	SB1060	■□□■■■■■□■■■■■□□■■■■■■■■■■■■■■■■■■■■■□□□□□	UC ?
B30	SB2651	■■□■■■■■□■■■■■□□■■■□■■■■■■■■■■■■■■■□■■□□□□□	ALG
Bv31	SB2651	■■□■■■■■□■■■■■□□■■■□■■■■■■■■■■■■■■■□■■□□□□□	ALG
Bv32	SB2651	■■□■■■■■□■■■■■□□■■■□■■■■■■■■■■■■■■■□■■□□□□□	ALG
B33	SB2651	■■□■■■■■□■■■■■□□■■■□■■■■■■■■■■■■■■■□■■□□□□□	ALG
Bv34	SB2651	■■□■■■■■□■■■■■□□■■■□■■■■■■■■■■■■■■■□■■□□□□□	ALG
Bv35	SB2651	■■□■■■■■□■■■■■□□■■■□■■■■■■■■■■■■■■■□■■□□□□□	ALG
Bv54	SB2651	■■□■■■■■□■■■■■□□■■■□■■■■■■■■■■■■■■■□■■□□□□□	ALG
Bv55	SB2652	■■□■■■■■□■■■■■□□■■■■□■■■■■■■■■■■■■■■□■■□□□□□	ALG
Bv56	SB2652	■■□■■■■■□■■■■■□□■■■■□■■■■■■■■■■■■■■■□■■□□□□□	ALG
Bv64	SB2652	■■□■■■■■□■■■■■□□■■■■□■■■■■■■■■■■■■■■□■■□□□□□	ALG
Bv65	SB2652	■■□■■■■■□■■■■■□□■■■■□■■■■■■■■■■■■■■■□■■□□□□□	ALG
Bv66	SB2653	■■■■□■■■■■■■■■■■■■■■□□■■■■■□■■■■■□■■□□□□□	ALG
Bv70	SB2653	■■■■□■■■■■■■■■■■■■■■□□■■■■■□■■■■■□■■□□□□□	ALG
Bv77	Orph 1	■■□□□■■■□■□■□■□□■■■■■□■■■■■■■■■■■■■■■□□□□□	Orphan 1
Bv78	Orph 2	■■□□□■■■□■□■□■□□■■■■□□■■■■■■■■■■■■■■■□□□□□	Orphan 2
Bv79	Orph 3	■■□■■■■■□■■■■■□□■■■■■■■■■■■■■■■■■□■■■■□□□□□	Orphan3
Bv80	Orph 4	■■□■■■■■□■■■■■□□■■■□■■■■■■■■■■■■■■■■■□□□□□	Orphan 4
Bv81	Orph 5	■■□■■■□□□■■■■■□□■■■□■■■■■■■■■■■■■■■□■■■■□□□□□	Orphan 5
Bv84	Orph 6	■■□■■■□■□■■■■■■□■■■□■■■■■■■■■■■■■■■□■■■■□□□□□	Orphan 6
Bv85	Orph 7	■■□■■■■■□■■■■■□■■■■□■■■■■■■■■■■■■■□□□□■■□□□□□	Orphan 7

SB: Spoligotyping Bovin, FRA: France, MEX: Mexico, ITL: Italy, BLG: Belgium, SPA: Spain; UC?: Unknown country (spoligotyping registered in the bovis database with unknown country), ALG: Algeria, Orph: Orphan.

Figure 4. Diagram of spoligotyping of *MTBC* strains isolated from cattle in *post-mortem*

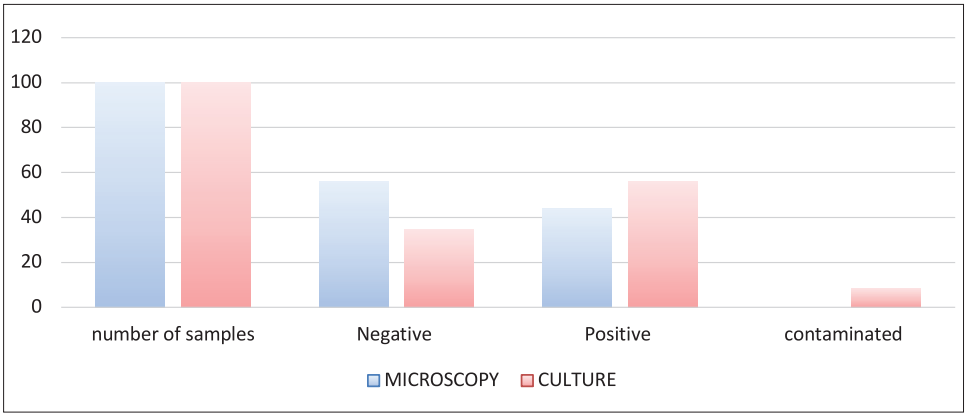


Figure 3. Bacterial culture for suspicious BTB cases

Typing by the Spoligotyping technique

The molecular identification of the 40 strains typed revealed the absence of spacers characteristic of *M. bovis* (3, 9, 16 and 39 to 43), except for two strains SB2653 which were devoid of spacers 5, 23, 24, 30, 36 and 39 to 43 (Figure 5). The analysis of the profiles found 19 different spoligotypes, divided into 8 clusters (the number of strains per cluster varied between 2 and 5), 4 isolated cases and 7 orphan profiles not yet listed in the World Bank.

The results of spoligotypes of *MTBC* strains isolated in cattle from the slaughterhouses of Algiers and Blida are shown in Figures 4 and 5.

According to Figures 4 and 5, of the 19 spoligotypes identified in which study, some profiles were similar to those reported in European countries (France, Belgium, Italy, Spain). One profile was identical to one reported previously in Mexico. Spoligotypes **SB1060** and **SB0120** were identified in local cows of Algerian origin, these same profiles were reported in the database though the origin of the country remains unknown. Previously unreported *M. bovis* spoligotype patterns have been named SB2651, SB2652, and

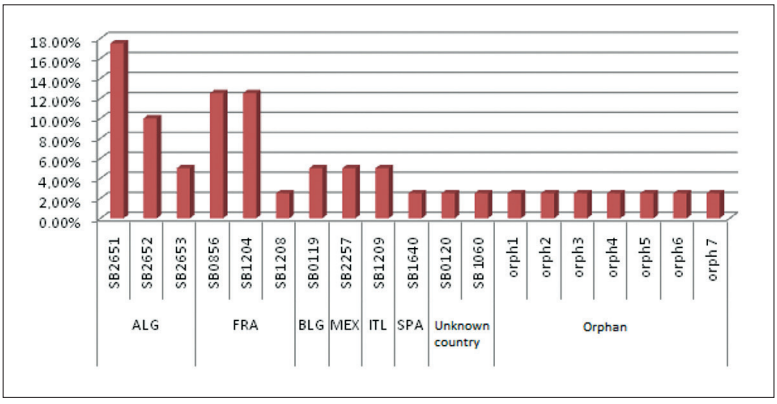


Figure 5. Percentage of bovine spoligotypes of *MTBC* strains isolated from *post-mortem* cattle

SB2653 by http://www.Mbovis.org, and are registered as Algerian spoligotypes.

Discussion

In the current study, 100 of 1300 bovine carcasses (7.69%) inspected presented BTB-like lesions. This result differs greatly from other reports. In Cameroon, of 129,165 slaughtered cattle inspected, 599 (0.46%) showed suspected TB lesions among a total of 983 (0.76%) identified pathologies (Awah-Ndukum et al., 2012). In Burkina Faso, the overall prevalence of BTB suspected lesions was 2.7% (58/2165) (Kanyala et al., 2022). In eastern Algeria (Bejaia), a retrospective abattoir study from 2009 to 2018 revealed an overall prevalence of 2.06% (4092/199,077) in cattle (Ayad et al., 2020). These differences could be explained by many factors including lower cattle density and housing of animals in open areas, which are unlikely to favour the spread of the disease, breed of animals slaughtered in the abattoirs, differences in the disease status in the animal populations, and different environmental influences (Regassa et al., 2010).

According to the results of this study, the frequency of infection increases with age. This can be explained by the nature of the disease which has a chronic course and the possibility of exposure to infection increases with time, which is why the disease occurs frequently in older animals. Our results differ from those reported by Tazerart et al. (2021) who found that younger cattle were the most affected due to the nature of the samples, which were isolated mainly from animals of this age group (less than 2 years).

In this study, females were more affected than males. Our data are in agreement with previous reports (Mimoune et al., 2022). These results can be explained by several factors, in particular that during the fattening period, in most cases, the animals (mainly males) prepared for slaughter are raised in an intensive system. In addition, depending on the ability of breeders and veterinarians who monitor the breeding, these animals are treated with antiparasitics and antibiotics against infectious diseases, as well as other substances such as vitamins, which leads to a decrease in the number of diseases (Hamiroune et al., 2020).

The prevalence of the disease was high in animals of the local breed compared to those of other breeds, in agreement with a previous study (Mimoune et al., 2022). Local breeds (mainly females) are reared extensively (unlike other breeds) which promotes the transmission of infectious diseases in the case of the presence of survival factors and the development of contamination agents.

In the current study, lesions were more frequently observed in the lungs and the liver, which is in agreement with previous reports (Cardoso et al., 2009; Silva et al., 2018). The chronic nature of the disease (explaining the spread of TB from lungs to other organs) has been attributed to the long incubation period of the disease, and acquired and maternal immunity (Oloya et al., 2006; Awah-Ndukum et al., 2012).

Direct smear examination revealed 44% positive slides. These results are not surprising since the microscopic examination is not sensitive and it is only positive if the sample contains 5,000 to 10,000 bacilli/mL (Proano-Pérez et al., 2011). Our results are in accordance with reports by Sulieman and Hamid (2002) with a positivity of 64 of 120 lesions collected in slaughterhouses in Sudan (53.3%). In another study, Varello et al. (2008) analysed 173 suspected tissue samples from bovines and identified 117 of them (67.63%) as tuberculosis using hematoxylin-eosin staining, but when Ziehl-Neelsen stain-

ing was used, AFB was identified in only 31 samples (17.91%).

Despite all lesions having characteristics of tuberculosis lesions, bacterial isolation was not possible in all of them due to some restrictions such as a low quantity of AFB in samples or even difficulties related to the high level of natural contamination. From 100 cultures tested, 56 were positive. It is remarkable that the decontamination protocol and the state of the samples have an important role in the results of the culture, more so if the sample is fresh and not frozen and the time of contact with NAOH during decontamination, with a higher possibility that the bacilli will grow. Our results are higher than those reported by Proano-Perez et al. (2011) (36.4%). The bacterial culture revealed the 9% of cultures were contaminated. In a recent study by Kanyala et al. (2022), culture revealed the growth of *Mycobacterium* species from 77.6% (45/58) specimens. The remaining 13 cultures were either contaminated showing growth of other bacteria and yeasts (1.7%, 1/58) or negative (*i.e.*, no mycobacterial growth could be observed in any of the five cultures) (22.4%, 13/58).

Microscopically positive smears were found to be positive by culture and 12 negative smears were found to be positive by culture, which could be related to the sensitivity of this diagnostic method.

To the extent of our knowledge, there are few studies on molecular characterization of *M. bovis* strains isolated from cattle in Algeria. This molecular technique allows for both identification and epidemiological studies (genetic diversity (different spoligotypes)), and the study of transmission. It should be noted that the frequency of different spoligotypes of *M. bovis* detected did not differ markedly between the three slaughterhouses located in Algiers and Blida.

In total, 19 spoligotypes were identified in the present our study. The **SB0856** profile, previously reported in France, was isolated in five imported cows. The **SB1204** profile, previously reported in France, was isolated in five imported cows. The **SB0119** profile, previously reported in France, was isolated in two imported cows. The **SB0818** profile, previously reported in France, was isolated from one imported cow. The **SB0120** profile, previously reported in Belgium, was isolated in two imported cows. The spoligotype **SB1640**, previously reported in Spain, was isolated in one local cow. The profiles **SB1209** and **SB2257** were identified in four crossbred cows and were reported in Mexico and Italy, respectively.

The spoligotypes **SB1060** and **SB0120** were identified in local cows of Algerian origin, and these same profiles were reported in the database but with an unknown country of origin.

Previously **unreported** *M. bovis* spoligotype patterns were named SB2651, SB2652, and SB2653 (by http://www.Mbovis.org), and are registered as Algerian spoligotypes. SB2651 was identified in seven cattle, this profile is characterized by the absence of spacers 3, 9, 15, 16, 20, 36 and 39-43. SB2652 was identified in four cattle, and is characterised by the absence of spacers 3, 9,15, 16, 21, 36 and 39-43. SB2653 was identified in two cattle, and is characterised by the absence of spacers 5, 23, 24, 30, 36, and 39-43.

Of the 19 spoligotypes of *M. bovis* detected, 20 strains, representing 50% of all strains isolated were previously detected among strains isolated from French cattle (SB0856, SB0121, SB1204, SB0818). The three types of spoligotypes most frequently detected in Algeria (SB0856, SB0121, SB1204) are also the three most frequent types observed in France and are also found in other continental European

countries. The importation of live, infected but non-diseased animals of European origin into Algeria is likely the major cause of transmission of foreign strains to the Algerian cattle population. This is also the case for spoligotypes SB1209, SB0119 and SB1640 of Italian, Belgian and Spanish origin found in our study.

It should be noted that the spoligotypes SB2651, SB0856, SB1204 and SB2652 were found in the following percentages: 17.50%, 12.50%, 12.50% and 10.00%, reflecting the inter-animal transmission of these strains. Confirmation by another more discriminating technique such as MIRU-VNTR would be desirable. In another study performed in northern Algeria, in a total of 115 patients with TB, 107 strains were identified of *M. tuberculosis*, seven *M. bovis* and one "*M. pinnipedii-like*", while for bovine samples (181 TB-like lesions), 174 isolates were identified as *M. bovis*, three as *M. caprae*, three as "*M. pinnipedii-like*" and one as "*M. microti-like*". The majority of isolates (89.2%) belonged to 72 different SB (Damene et al., 2020).

Conclusion

Tuberculosis in Algeria is a major problem on cattle farms, causing significant economic losses. Many factors can be associated with the disease (age, sex, and breed). Laboratory techniques including microscopy and culture, supplemented by molecular identification, help to identify the origin of BTB. Spoligotyping of isolated strains enabled us to identify and confirm the diagnosis of BTB, to determine the various genomic profiles circulating among the animal herd, and to highlight new genomic profiles.

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Baciloskopija, bakterijska kultura i spoligotipizacija *Mycobacterium bovis* sojeva izoliranih u goveda iz sjevernosredišnjeg Alžira

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Cilj ove studije bio je procijeniti slučajeve slične tuberkulozi goveda u tri klaonice u sjevernosredišnjem Alžiru i potvrditi sumnjive slučajeve uporabom mikroskopije, bakteriološkog pregleda i tehnike molekularne biologije. Uočili smo čimbenike koji su utjecali na prevalenciju bolesti. Određeni su i genomski profili sojeva *Mycobacterium tuberculosis* kompleksa (MTBC) izolirani tehnikom tipizacije oligonukleotida (spoligotipizacija). Na razini klaonice, rutinski su pregledana trupla goveda u svrhu detekcije vidljivih abnormalnosti uključujući sumnjive lezije tuberkuloze goveda (BTB). Na razini laboratorija (Pasteur institut, Alžir), obavljeno je Ziehl-Neelsen bojanje, bakteriološka kultura, biokemijska studija (ispitivanje redukcije nitrata) te spoligotipizacija za potvrđivanje sumnjivih slučajeva. Od ukupno 1300 trupla goveda, 100 ih je pokazivalo stanja slična na BTB (7,69 %). Životinje starosti više od 5 godina bile su pogođenije u usporedbi s drugim dobnim skupinama. Ove su lezije zamijećene više u ženki nego u mužjaka, a goveda lokalne pasmine bila su najizloženija stanja slična na BTB (P<0,05). Podatci baciloskopije bili su pozitivni za 44 od ukupno 100 sumnjivih mužjaka (44 %), dok je bakteriologija pokazala da je 56 kultura bilo pozitivno, a 35 negativno, a 9 kultura bilo je kontaminirano. Molekularna spoligotipizacija 40 uzoraka sojeva *Mycobacterium* pokazala je 19 profila spoligotipa *M. bovis*, 50 % profila već je detektirano u mediteranskoj regiji, a 3 obrasca spoligotipa koji do sada nisu prijavljeni, nazvani su SB2651, SB2652, SB2653 od strane http://www.Mbovis.org. Zaključno, potrebno je preporučiti mjere i sredstva za prevenciju prijenosa TB među životinjama i na ljude, kao i potrebu za intenzivnim istraživanjima uporabom rutinskih i molekularnih dijagnostičkih tehnika za razumijevanje i istraživanje MTBC.

Ključne riječi: *tuberkuloza goveda, baciloskopija, kultura, spoligotipizacija, prevalencija, faktori*