Prevalence of Bovine Tuberculosis Gross Lesions in Doukkala slaughterhouses, Morocco

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Doi: 10.19044/esj.2019.v15n18p38 URL:http://dx.doi.org/10.19044/esj.2019.v15n18p38

Abstract

In Morocco, tuberculosis (TB) is a major zoonotic disease with very serious hygienic and economic impacts. Our study aimed to investigate bovine tuberculosis in two Moroccan slaughterhouses in Doukkala area based on the observation of macroscopic gross lesions. 4198 cattle were examined for tuberculosis lesions during the period of end of May 2014 to July 2015. The results showed that 64.4% of cattle with TB lesions were male with a dominant age of less than or equal to 2 years (67.2%). Females represented only 35.6% with a dominant age between 4 and 8 years (62.3%). The dominant breed was the cross breed of imported and local breeds with 95.4%. Tuberculosis-like lesions were observed in 194 animals (4.6%) including 10 (5.15 %) of generalized and 184 (94.85 %) of localized lesions, mainly encountered in the lungs (44.83%), the head lymph nodes (42.32%) the liver and its associated lymph nodes (9.41%). An estimated overall daily prevalence of 4.6% and a daily average of 5.4% (95% CI: 4.7 - 6.1%) were noted. Compared data obtained from the slaughter database 1.97 % (95% CI: 1.8-2.4), this prevalence is more than twice of estimated average (RR=2.34). This assumes that routine inspection leaves more than 50% of animals with suspicious lesions. On the other hand, 390 gross lesions were collected for culturing. An isolation frequency of *Mycobacterium* of 85.6% was obtained and the LPJ and Herrold seem to be the best culture media. **Keywords:** Bovine tuberculosis, post-mortem, gross lesions, prevalence, Culture, Mycobacterium, Slaughterhouses, Morocco

Introduction

Bovine tuberculosis (BTB) is a chronic infectious disease caused by *Mycobacterium bovis* (*M.bovis*). It is widely distributed throughout the world and could be transmitted to human, which constitutes public health concern (Ameni and Erkihun, 2007). The transmission of BTB to human could occur via the consumption of unpasteurized infected milk and infected meat (Cousins et al., 2001). The respiratory route is considered to be the primary mode of infection within cattle (Morris et al., 1994). BTB infection is also transmissible from cattle to humans directly by aerogenous route and through direct contact with material contaminated by nasal and oral secretions from infected animals (WHO, 1994), meanwhile occupational infection may occur among farmers, veterinarian, and slaughterhouse workers (Biet et al., 2005). The global prevalence of human tuberculosis caused by *M. bovis* has been estimated to be 3.1% of all tuberculosis cases, accounting for 2.1% and 0.4% of mulmonary and entry multiple occupation is cased.

The global prevalence of human tuberculosis caused by *M. bovis* has been estimated to be 3.1% of all tuberculosis cases, accounting for 2.1% and 9.4% of pulmonary and extra- pulmonary tuberculosis cases respectively (Cosivi et al., 1998). The prevalence of *M. bovis* among human TB cases is low in the developed world (O'reilly and Daborn, 1995; Whipple et al., 1996) but its importance in most of the developing countries is unknown and could be high due to a lack of control programmes and the high prevalence of BTB (Neill et al., 1994).

Zoonotic TB can also be considered a socio-economic disease; it has a negative impact on livestock production in developing countries through reduced production efficiency, carcass or organ condemnation and restriction of international trade. The detection of infected animals is mainly limited to the routine slaughterhouse inspection.

In Morocco, bovine tuberculosis has been reported in cattle since 1939 as a result of importation of cattle from Europe and the cases have increased with intensification of dairy livestock (Fikri, 1997). Officially, tuberculin skin and ELISA tests and slaughter inspection are the current control strategy applied in Morocco. However, due to budget constraints for breeder compensation, there is no systematic BTB screening of cattle at a national level (ONSSA, 2017).Live animal teting is limited to medium and large cattle dairy herds.

Identification of M. *bovis* by culture and molecular methods is important for a definitive diagnosis (Corner et al., 2012). As the slaughterhouses in Morocco do not have diagnosis facilities for rapid confirmation of detected BTB macroscopic lesions, the visual inspection of carcasses and offal remains a usual the only technique for monitoring the prevalence of BTB in Morocco.

It's within this framework that our study is undertaken to achieve the following objectives:

- Assess the prevalence of BTB in slaughterhouses of Doukkala region (Morocco),
- Determine the epidemiological characteristics of the slaughtered cattle with evident BTB-like lesions,
- Use four media culture to isolate *Mycobacterium* from these lesions.

Materials and methods

The study was conducted in Doukkala's slaughterhouses (El jadida and Had Ouled Frej cities) from end of May 2014 to July 2015. Doukkala region has enormous potential to be a preferred destination for businessmen wishing to invest in the agro-industrial sector. Thanks to its important road and rail infrastructure, in addition to its proximity to three ports and major urban centers (Casablanca and Marrakech among others), this region has become a real national agro-industrial hub. The agricultural sector in the Doukkala region contributes up to 10% of the national agricultural Gross Domestic Product (GDP) and to 22% to regional GDP. The dairy sector is among the most productive sectors in the region with a total of 147,000 dairy cows (95% improved breeds) and 43,200 dairy farmers. Doukkala holds also large markets where animals from different region of Morocco and different ages and breeds are mixed and brewed, thus facilitating diseases transmission including tuberculosis. Meat inspection is governed by regulations that provide for *ante-mortem* and *post-mortem* examinations. It is carried out by assigned meat veterinary inspectors in each abattoir. The procedures employ meat inspection protocols issued from Moroccan health veterinary authorities. They involve visual examination and palpation of organs such as the lungs, the liver and kidneys, in addition to palpation of organs such as retropharyngeal, tracheobronchial, mediastinal and other lymph nodes. Further examinations of other lymph nodes and organs, or the whole carcass are performed whenever lesions are detected in one of these tissues. The whole carcass is condemned in generalized BTB, associated with cachexia involving multiple lymph nodes, while the whole organs (or their parts) are discarded if large tuberculous lesions are found in their parenchyma or associated lymph nodes. When gross lesions of BTB are found in any of the tissues, the animal is classified as having lesions (BO, 2001).

In our study, 4,198 cattle slaughtered were examined by visual and physical inspection of external and internal organs under sufficient light, with special focus on systemic portal lymph nodes for possible presence of nodules. 319 samples of gross lesions suggesting BTB were collected from

194 infected animals in sterile plastic containers and were conveyed to Hassan II Agronomic and Veterinary Institute in Rabat and stored at -20 °C for *Mycobacterium* culture purpose (Table 1).

Sample nature	Number	Percentage (%)
Retro-pharyngeal lymph nodes	129	(66.5)
Pulmonary lymph nodes	89	(45.9)
Pulmonary parenchyma	54	(27.8)
Hepatic parenchyma	20	(10.3)
Retro-hepatic lymph nodes	10	(5.2)
Mandibular lymph nodes	6	(3.1)
Pleural lesion	4	(2.1)
Mesenteric lymph nodes	2	(1.0)
Renal lesion	2	(1.0)
Iliac lymph nodes	1	(0.5)
Pre-scapular lymph nodes	1	(0.5)
Pericardial lesion	1	(0.5)
Total	319	

 Table 1: Distribution of the samples among BTB cases as recognized by gross lesions examination

The samples were processed and cultured according to the method described in the "Laboratory Methods in Veterinary Mycobacteriology" manual (United State Department of Agriculture, 1985). The samples from each animal having shown suspicious lesions of tuberculosis were treated according to the method described above and pooled with approximately 5 g of tissues to obtain a ground material that will serve as an inoculum.

For each inoculum, two different culture media were used excepting two samples for witch three media were used. Cultivation involved samples from 194 animals in a total of 390 culture tubes. The media used for the primary culture of Mycobacterium from BTB-like lesions were mainly eggbased media: Lowenstein-Jensen without glycerine (LJ), Lowenstein-Jensen with glycerine (LJG), Lowenstein-Jensen enriched with pyruvate (LJP) and Herrold. Each sample was seeded in two different media, especially in order to increase the chances of having mycobacterial colonies. Transplanting was carried out for any colony having shown the Acido-Alcohol-Resistant (AAR) character on the Proskauer and Beck (P & B) liquid medium (Proskauer and Beck,1898) and then on one of solid media. The detailed assessment of the cultivations according to the media used and the origin of the samples is presented in Table 2.

The combination of culture media that has been frequently used for each sample is that of LJP and Herrold media. When monitoring incubated cultures, suspicious colonies were systematically stained using the Ziehl-Neelsen staining (ZN) to check the AAR character of the bacilli. The results are noted to ensure the simple traceability. The sample is considered positive if the culture is confirmed positive in at least one of the used media .

An Excel MS Office database has been developed to ensure both data capture for each sample and crop monitoring data using the function of the "PivotTable Report". The analysis features of the same software were used for data analysis and results presentation.

Culture media	Number	Percentage (%)
LJ with sodium pyruvate (LJP)	144	(36.9)
Herrold (Herr)	106	(27.2)
Lowenstein Jenson (LJ)	89	(22.8)
LJ with glycerin (LJG)	51	(13.1)
Total	390	(100)

Table 2: Distribution of the media used for Mycobacterium culture

Results

A-Tuberculosis-like lesions

Results showed that 64.4% of cattle with macroscopic gross lesions were male with a dominant age of less than or equal to 2 years (67.2%). Females represented only 35.6% with a dominant age between 4 to 8 years (62.3%). The dominant breed was the cross bred animals originated from local or imported breedings with 95.4% (Table 3).

	ndividual characteristics of		· · ·	
Feature		Number	(%)	
Originated				
production	Dairy	174	(89.7)	
system	Beef	20	(10.3)	
	Female	69	(35.6)	
Sex	Male	125	(64.4)	
	Total	194	(100)	
	Females		. ,	
	≤ 2 years	8	(11.6)	
	2 to 4 years	7	(10.1)	
	4 to 8 years	43	(62.3)	
	> 8 years	11	(15.9)	
	Subtotal	69	(100)	
Age class	Males			
	\leq 2 years	84	(67.2)	
	2 to 4 years	41	(32.8)	
	Subtotal	125	(100)	
	Total	194	(100)	
	Cross breed	185	(95.4)	
Breed	Imported purebred	9	(4.6)	
	Total	194	(100)	

Taking into account the origin of BTB cattle sampled, we noted that they come mainly from Markets close to the town of El Jadida namely Sidi Bennour (36.6%) and Had Ouled Frej (28.9%) (Table 4).

Table 4: Reported origin of tuberculosis cattle cases based on gross lesions.					
Declared origin	Cattle ni	Cattle number %			
Sidi Bennour	71	(36.6)			
Had Ouled Frej	56	(28.8)			
Sebt Douib	44	(22.7)			
Other areas	23	(11.9)			
Total	194	(100)			

The majority of the macroscopic lesions observed on tuberculous bovine carcasses were detected at the level of the ribcage (44.83%) and concerned the lungs and bronchial lymph nodes, as well as at the level of the head, mainly in the retro-pharyngeal lymph nodes (42.32%) and also the liver and its lymph nodes (Table 5 and Photo 1).



Photo 1: Tuberculous lesions in different organs of cattle in Doukkala area. a-Heart (pericardium), b-Retropharyngeal nodes c-Lungs, d-Liver

Table 5: Localisation and frequencies of tuberculosis lesions					
Location of lesions	Grosse BTB lesions				
	Number	(%)			
Lungs*	143	(44.83)			
Head [*]	134	(42.32)			
Liver*	30	(9.41)			
Pleura	4	(1.25)			
Kidneys	2	(0,63)			
Mesenteric lymph nodes	2	(0.63)			
Pericardium	1	(0.31)			
Prescapular lymph nodes	1	(0.31)			
Iliac lymph nodes	1	(0.31)			

*Organ and lymph nodes

Among observed progressive tuberculosis forms, the stabilized primary complex is reported in 80.4% of cases followed by stabilized nodular tuberculosis with 10.8% as mentioned in Table 6.

Evolutionary stage	Number	(%)
Stabilized primary complex	156	(80.4)
Chronic organ tuberculosis	8	(4.1)
Generalized tuberculosis	7	(3.6)
Stabilized nodular	21	(10.8)
tuberculosis		
Absence of lesions *	2	(1.1)
Total	194	100

Table 6: Frequencies related to the progressive stage of bovine tuberculous lesions.

Out of 4,198 cattle carcasses inspected, 194 (4.6%) showed tuberculosis-like lesions. Generalized tuberculosis forms were found on 7 cases (3.6%), mainly in cattle of over 4 years old. Organ partial seizures involved 184 carcasses (94.8%) while 10 whole carcasses were seized (5.2%) depending on the progressive forms of tuberculosis, the general body condition of the animal and affected organs. The main organs seized were the head and lungs, with respectively rates of 63.4% and 44.8% cases (Table 7).

Table 7: Distribution of organs partial seizures.					
Organ	Cases %				
Head	123 (63.40)			
Lung	87 (44.84)			
Heart	86 (44.32)			
Liver	23 (11.85)			
Pancreas	6 (3)				
Total	325				

According to the slaughterhouse data recorded, in 753 days of slaughter during the period from September 1st 2013 to October 31st 2015, 26,060 cattle were slaughtered, of which 514 showed lesions of tuberculosis like lesions. The overall daily prevalence was estimated to 1.97% and the daily

average to 2.1% with a 95% confidence interval of 1.85 to 2.4.During the study period from June 20th, 2014 to July13th 2015; while working under sufficient light and giving enough time to inspect each carcass; out of 4,198 cattle examined according to the regulatory protocol, 194 carcasses showed suspicious lesions of tuberculosis giving an estimated overall daily prevalence of 4,6% and the daily average at 5,4% (95% CI: 4.7-6.1%) (Table 8). This value, compared to that obtained from the slaughterhouses registered data is more than twice the estimated average (RR=2.34). This assumes that routine inspection leaves more than 50% of animals with suspicious lesions.

	Study period	Slaughtered cattle number	Cattle with like lesions number	Global prevalence (%)	Daily average prevalence	confidence interval IC (95%)	Min - Max	RR (relative risk)
Slaughterhouse records	753 day [*] (09/09/13 - 10/31/15)	26060	514	1,97	2,1	1,8 to 2,4	0 - 50	
Slaughterhouse study	110 day [*] (05/26/14 - 07/13/15)	4198	194	4,6	5,4	4,7 to 6,1	1,1 - 25	2,34 (1,99 – 2,75)

 Table 8: Observed and estimated daily average prevalences

*: during these periods only working days were taken into acount

B-Results of *Mycobacterium* cultures

Globally among 390 samples, 279 (71.5%) showed a positive mycobacteria culture. Depending on the medium used, the relative frequency of positive isolates per culture is shown in the Figure 1. The latter shows that the LJP medium offers the best isolation result of mycobacteria with a success rate of 80.5%, followed by the Herrold medium 78.3%.

In our study, out of 194 cattle with tuberculous like lesions, 166 showed a positive result in the *Mycobacterium* isolation, which means an isolation frequency of 85.6%.



Figure 1: *Mycobacterium* isolation frequency according to the culture medium Lowenstein-Jensen without glycerine (LJ); Lowenstein-Jensen with glycerine (LJG); Herrold; Lowenstein-Jensen enrichied with pyruvate (LJP).

Discussion

The majority of tuberculous cattle are from dairy-type breeds. This shows the lack of specialization of farms where a dairy genetic potential can be sacrificed in slaughterhouses. Dairy farming has long been one of the priority axes of agricultural policies in Morocco. According to Benlekhal et al. (2012), improved breeds (pure and crossed) constitute 82% of cattle breeds in irrigated areas. It's the case of the study area.

In this study, BTB gross lesions were found most frequently in the lungs and lymph nodes of thoracic cavity, followed by lymph nodes of the head. These findings indicate that inhalation might be the principal route of BTB infection in cattle. Whipple et al. (1996) has reported that 60% of tuberculous lesions were localized in thoracic lymph nodes versus 26.7% in head ones. The same findings were observed in Australia (Tammemagi et al.,1973).In Mexico, according to Miliano-Suazo et al. (2000), 49.2% of lesions involved the retropharyngeal lymph nodes. In Algeria, Sahraoui et al.(2008) mentioned that the distribution of injuries showed a violation of respiratory lymph nodes with a rate of 76.92%.Furthermore, Corner (1994) had reported that up to 95% of cattle with BTB gross lesions could be identified by examination of the lung and associated lymph nodes in agreement with other studies (Whipple et al., 1996; Pritchard, 1988 ; Teklu et al., 2004).

The predominance of the stabilized primary complex could be explained by the young age of the animals slaughtered, as 79% of cases with this form are less than 4 years old.

Of the 194 carcasses inspected, forms of generalized tuberculosis were noted in 7 (3.6%) of them mainly in cattle over 4 years of age. According to

Nassar et al.(1994) in Brazil, Demelash et al., (2009) in Ethiopia, Aljameel et al.(2014) in Sudan and Youssef and Ahmed, (2014) in Egypt, the generalized form represents between 2.5 and 20.5% of cases of seizure at slaughterhouse level.

The overall daily prevalence of 4.6 % found in the current study is higher compared to the findings of previous studies performed with similar diagnosis methods in Moroccan slaughterhouses. According to the national office of food safety (ONSSA), bovine tuberculosis is rife in Morocco in the enzootic state in cattle farms. Indeed, this disease is regularly observed in national slaughterhouses (with an average prevalence of 1.7% recorded during the period 2000-2010) during veterinary inspections carried out by ONSSA veterinary inspectors (ONSSA, 2017). It is also greater than that of 1.97% calculated from local slaughterhouse database between September 2013 and October 2015. In fact, during our study period an attempt was made to take the time necessary to inspect all suspect carcasses under an appropriate lighting and by applying rigorously the inspection protocol. This shows that in a context known for the dilapidated state of infrastructure at the slaughterhouse level, where adequate lighting during night inspection, especially at the level of rural abattoirs, can sometimes be lacking, cases with insidious tuberculosis can escape to the veterinary inspection. In Ethiopia, Demelash et al. (2009), Mihreteab and Indris (2011) reported respectively 10,2 % and 2,7% as abattoir prevalence. In Mexico as well, meat inspection at slaughterhouses demonstrated a frequency of 16% of BTB lesions (Miliano-Suazo et al., 2000).In Algeria, Sahraoui et al. (2008) estimated the overall prevalence to 3.58%.

According to Demelash et al. (2010); Dechassa (2014); Mihreteab and Indris (2011), many factors may influence the effectiveness of the inspection at slaughterhouse ; such as the inspection method, the stage of the lesions, the lack of training of the inspection staff, the lack of a regular system for assessing the competence of meat inspectors, the workload (number of carcasses per inspector and inspection time per carcase), the failure of the slaughter infrastructure; for example, insufficient lighting during night inspections, the possible direct or indirect influence of butchers on the inspectors' decision, especially when the seized parts show little or no visible lesions. Such problems are often reported in developing countries where the access of butchers and workers is tolerated during meat inspection. In Morocco, there are 200 municipal and nearly 800 rural slaughterhouses. The province of El Jadida is home to fifteen rural and three municipal slaughterhouses including El Jadida, which produces more than 30% of the province's controlled meat. It is the only one to have an air handling system, while the others are in fact traditional, with a ground slaughter and preparation of the carcass on the spot. At the beginning of 2018, the veterinary services

opted for the delegation of veterinary inspection missions to the mandated sanitary veterinarians (private practitioners) to fill the lack of veterinary officiel inspectors and support the health control in the rural abattoirs. In most abattoirs in Doukkala region, animals are slaughtered during the night to provide consumers with fresh raw meat the next morning. In most rural slaughterhouses where the electric power supply doesn't exist or is insufficient, proper detection of BTB gross lesions may be difficult, consequently, more infected carcasses could escape to control. However, the prevalence of BTB-like lesions showed in this study is relatively lower as compared to the prevalence of the disease in dairy farms in Morocco. Indeed, the national survey, conducted in 2004, showed an individual cattle prevalence of 18% and a herd prevalence of 33% (FAO, 2011). This is also in line with other studies conducted in other countries indicating lower infection rates in beef than in the dairy cattle (Pritchard, 1988). The culture detected *Mycobacterium* in two cachectic cows that did not show *post-mortem* lesions. Results of various studies have indicated that in some cattle infected with *M. bovis* gross lesions are not visible in the tissues snow *post-moltem* lesions. Results of various studies have indicated that in some cattle infected with *M. bovis* gross lesions are not visible in the tissues examined at slaughter (Whipple et al., 1996; Teklu et al., 2004). Actually, the sensitivity of gross *post-mortem* examination is affected by the method employed and the anatomical sites examined (Corner, 1994).

The frequency of the samples used in this study depends on the progressive stage of BTB lesions observed during the inspection. The dominance of pulmonary and retropharyngeal lymph nodes and pulmonary and hepatic parenchyma reflects the high frequency of the primary complex thus the inhalation is the dominant route of infection.

Laboratory investigations applied to macroscopic lesions taken from 194 tuberculous cattle resulted in an overall 85.6% *Mycobacterium* isolation frequency. LJP and Herrold media with a success rate of 80.5% and 78.3%, respectively, obtained the best isolation results for mycobacteria. This is in agreement with the previously reported studies that reported both media promote the growth of *M. bovis* and that *M. bovis* is by far the most isolated on tuberculous lesions of cattle (Corner and Nicolacopoulos,1988; WHO, 1994: Corner et al. 2012) It would therefore be practical to appreciate the f 1994; Corner et al.,2012). It would therefore be practical to associate the two media for the following reasons: their ability to allow the growth of other species of mycobacteria, including *M. tuberculosis* and M.*avium* complex; their great sensitivity and characteristic aspect of the colonies; their cost price remains affordable; LJP enhances growth of *M. bovis* and *M. africanum* (increase in size and number of colonies) and the Herrold medium promotes the growth of the MAP by adding growth factors (Mycobactin).

Conclusion

The findings of the study showed that the slaughterhouse prevalence of bovine tuberculosis in the studied area seems higher. The comparison between the estimated overall daily prevalence and that obtained from the slaughter database assumes that routine inspection leaves more than 50% of animals with suspicious lesions. A large-scale surveillance program is needed to estimate the apparent and true prevalence of bovine tuberculosis. The laboratory isolation frequency of mycobacteria from TB lesions was 85.6%. The combination of LJP and Herrold media increases the chances of isolation of mycobacteria.

Acknowledgements

The authors are grateful to the Staff of Doukkala slaughterhouses for their good willing and valuable cooperation during the different stages of this study.

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