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EPIDEMIOLOGICAL ABROACH IN BOVINE TUBERCULOSIS BY SPOLIGOTYPING IN EGYPT

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ABSTRACT

Bovine tuberculosis is a chronic and contagious disease that affects domestic animals, wildlife, and humans. Caused by *Mycobacterium bovis*, Bovine tuberculosis causes major economic losses and poses a serious constraint to international livestock trade. In Egypt, although there is a national Bovine tuberculosis control program, epidemiological studies are crucial to identifying the source of bovine tuberculosis infection, and its transmission dynamics and host preference. This article considers a trial to give first epidemiological snap shot about Bovine tuberculosis situation in Egypt. By examining 100 samples collected from slaughterhouse surveillance studies conducted in 2016 in El-Bassatin slaughter house, Cairo and apply epidemiological tool (spoligotyping) in all DNA extract after culturing, all positive samples on culture and biochemical reactions give negative sample with spoligotyping approach.

Key words:

Mycobacterium bovis, cattle diseases, tuberculosis, spoligotyping, Egypt.

INTRODUCTION

Bovine tuberculosis is a chronic infectious disease caused by *Mycobacterium bovis*. This disease mainly affects cattle, but can also be found in other domestic and wild animals, and occasionally, in humans. The World Organization for Animal Health (OIE) considers BTB to be an important zoonotic disease with a socioeconomic and public health impact that affects the international trade of livestock and animal products [1]. The prevalence of BTB in developing countries remains largely unknown. According to a study conducted in 2006, Egypt among a group of African countries assumed to have a relatively high prevalence of Bovine tuberculosis, but there is a lack of reporting [2]. Other countries in the group are Argentina, Brazil,these countries considers Meat exported countries to Egypt.

This absence or lack of control represents a high risk for the rural inhabitants living in direct contact with animals [3]. In humans, the proportion of tuberculosis induced by *M. bovis* is relatively low compared to *M. tuberculosis*. In recent years, however, *M. bovis* tuberculosis in humans has become increasingly prevalent among human populations subjected to poverty, malnutrition,human immunodeficiency virus (HIV),and inadequate health care [4].

Transmission through the consumption of unpasteurized milk and dairy products from infected cattle occurs mostly among the general public,whereas exposure through airborne infection remains highest among farmers, veterinarians, and slaughterhouse workers (5). According to a study, *M. bovis* could be responsible for more than 2% of the total pulmonary tuberculosis (TB) cases and 9.4% of extra pulmonary forms among humans in Africa, a considerable number. In addition, another study showed that in Argentina, 2% of human TB cases have been recorded as being caused by *M. bovis* [2]. Given the appearance of new cases in recent years, Bovine tuberculosis in humans has been designated a reemerging disease in developed countries. Reemergence is most likely the result of increased world population, augmented by the movement of people and animals, environmental changes, crossing of the interspecies barrier, and changes in livestock production management [6, 7, and 8]. The total economic losses due to BTB are underestimated because its impact on public health has not been thoroughly evaluated [7]. loss are limited to animal health issues and are based on weight loss,decreased milk production, lower reproduction rates, mortality, and condemnation of carcasses [8].

In Egypt, there are few research groups working on Bovine tuberculosis, which explains the limited number of published studies. Bovine tuberculosis prevalence cases in Egypt are not documented nor quantified clearly for several reasons: lack of proper recording of positive cases, insufficient veterinary inspection in most slaughterhouses, but mainly because Bovine tuberculosis is not a notifiable disease. Furthermore, it is not unlikely that in Egypt, as may be the case in other countries, Bovine tuberculosis is not reported due to a lack of trust between farmers and health officials [3]. Currently,control measures related to relocation and transportation of animals focus solely on Foot and Mouth Disease. Bovine tuberculosis prevalence can be influenced by several factors at the individual, herd, provincial, or country level (9). For instance, livestock husbandry varies by herd size, farm size, and type of cattle industry, i.e., for dairy or meat production. Factors such as these can affect Bovine tuberculosis disease

occurrence by increasing or decreasing close contact among animals. In Egypt, the cattle population is not equally distributed, this variation of distribution also impacts Bovine tuberculosis occurrence. In geographic areas with high Bovine tuberculosis, prevalence rates can be estimated by the proportion of macroscopic tuberculous lesions detected during postmortem examination (followed by the rejection of carcasses and viscera from these animals), if a reliable system exists [7]. Indeed, programs based on slaughterhouse surveillance are only effective when they use a reliable traceability system for tracing-back to herd of origin. The distribution and development of lesions depend on the route of transmission [10], and location can vary, although most often they are found in thoracic lymph nodes due to infection via the respiratory route [11]. On the other hand, detailed meat inspection allows the identification of lesions in apparently healthy animals, which increases the number of detected animals and avoids the consumption of Bovine tuberculosis-infected cattle [13].

This article aims to provide information on the current situation of Bovine tuberculosis in Egypt based on available data from studies of veterinary inspections at El- Bassatin slaughter house, and laboratory-based diagnoses.

MATERIAL AND METHODS

Sampling out of 100 samples collected from El-Bassatin slaughter house during 2016. Culturing of all collected samples was done on LJ medium and stone brink followed by biochemical reaction (Arylsulfatase, PNB, Niacin, TCH and Catalase).

DNA isolation To obtain genomic DNA for spoligotyping, mycobacterial colonies freshly grown on LJ medium were re-suspended in 200l of tris-ethyl enediaminete traacetic acid (EDTA) buffer (10 mM Tris-HCL, 1 mM EDTA [PH 8.0]), followed by heat inactivation at 100°C for 5 min and centrifugation at 10,000 g for 15 s to pellet cell debris. The supernatant, containing DNA, was stored at - 20C and used in polymerase chain reactions (PCRs.).

Amplification of the spacers is accomplished by using the primers DRa and DRb, which enable to amplify the whole DR region. Only a very small amount of template DNA is required. Typically the PCR is performed on 10 ng purified chromosomal Mycobacterial DNA but, with minor adaptations, DNA extracts from lysed bacteria (from freezer or Löwenstein) can serve as template. The PCR products are labeled with biotin, because primer DRa is biotinylated.

Always include chromosomal DNA of *M. tuberculosis* strain H37Rv and *M. bovis* BCG P3 as positive controls. Use water as a negative control then Prepare the reaction mixture 2 µl template DNA 3 µl primer DRa (0.2 µmol/µl) 3 µl primer DRb (0.2 µmol/µl) 20 µl 2×Taq PCR Master Mix 12 µl MQ water (to a final volume of 40 µl) 3. Place the tubes in a PCR-apparatus for amplification, and perform the following temperaturecycling3 min 94°C 1 Cycle 1 min 94 °C 1 min 55 °C 30 sec 72 °C 25 Cycles7 min 72°C 1 Cycle 4°C.

Spoligotyping was performed using primers DRa and DRb, corresponding to DR region of the MTBC genome, according to the procedures described by **(Kamerbeek *et al.*, 2001).** Amplification and hybridization were performed using a membrane prepared in‐house. Detection of hybridized DNA was achieved using enhanced chemiluminescence (ECL) detection liquid (Amersham Biosciences) followed by exposure to X‐ray film (hyperfilm ECL; Amersham.

RESULTS

Through the slaughterhouse sampling, all 20 positive cultures obtained from 100 tissue samples of tuberculous-like lesions were identified; median time to culture positivity was 26 days. After the bacteriological examination using conventional cultivation method on solid medium (LJ medium and stone brink).All isolates were found to be positive slow-growers *Mycobacterium* species showing rough, crumbly, waxy and non-pigmented (cream colored) colonies ,biochemical reaction results were positive for Arylsulfatase , negative for PNB negative for Niacin, negative for TCH and negative for Catalase.

DNA-fingerprinting analysis allowed identifying different *M. bovis* strains. All *M. bovis* isolates from El - Bassatine slaughter house display negative results by spoligotyping technique.

DISCUSSION

The design of intervention strategies in animals is informed mainly by the epidemiology of the disease. Comprehensive epidemiological studies of bTB can provide valuable insights into the sources of infection, routes of transmission, geographical localization, host preference, disease dynamics and risk factors for the maintenance and spread of the disease, thus contributing to contain the disease in animals and reduce the risk to humans [14].

Various molecular-based techniques have been developed to study the epidemiology

of *M. bovis* infections [15]. Spoligotyping [16] and mycobacterial interspersed repetitive unit-variable number tandem repeats (MIRU-VNTR) typing [17, 18] are the most commonly used methods for *M. bovis* genotyping. The use of spoligotyping in combination with MIRU-VNTR typing has been shown to improve the discriminatory power of *M. bovis* typing [19-20]. Combined genetic profiles have recently been used to analyze the transmission of *M. bovis* in France [21], Midwest Brazil [22], Cameroon [23] and Mozambique [24], most of these countries considers as exported countries for slaughtered and live animals for meat consumption to Egypt.

The study applied various laboratory based diagnostic methods,in addition to the macroscopic investigation. The presence of *M. bovis* was confirmed [14]. Also the few No. of isolates collected give a concise picture about the situation of BTB in Egypt during 2016, and that consider main reason for all negative results, so, recommended to apply the study on a large scale of slaughter houses all over Egypt that helping and giving a clear picture about epidemiological situation of BTB in Egypt.

CONCLUSION

The presence of *M. bovis*, and a lack of Bovine tuberculosis controls, has caused a rise in Bovine tuberculosis prevalence and consequently, a growing push for the implementation of epidemiological national Bovine tuberculosis program.

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