Kafrelsheikh University

Faculty of Veterinary Medicine
Animal Medicine Department
(Infectious Diseases)

Comparative Diagnostic Study on Tuberculosis in Cattle and Buffaloes

A thesis submitted

By

Mohamed Ahmed Ali Borham

B. V. Sc., Kafrelsheikh University, 2011
M. V. Sc., Kafrelsheikh University, 2016

For

The degree of Ph. D. in
Vet. Medical Science
(Infectious Diseases)

Under Supervision of

Prof. Magdy H. Al-Gaabary
Prof. of Infectious Diseases and Head of Animal Medicine Department
Faculty of Veterinary Medicine
Kafrelsheikh University

Prof. Attia A. El-Gedawy
Chief Researcher of Bacteriology
Animal Health Research Institute
Dokki

Dr. Atef F. Oreiby
Assistant Prof. of Infectious Diseases
Faculty of Veterinary Medicine
Kafrelsheikh University

2021

Contents

Contents

Contents

List of Tables

Table…….... Page

List of Tables

List of Figures

Figure……...Page

List of Figures

List of Figures

List of Figures

List of Figures

List of Figures

Summary

This study was conducted at the Middle-Delta region; Disuk, Sidi-salem, EL-Reyad, Baltim and Biyala districts of Kafrelsheikh Governorate as well as Tanta abattoir at Gharbia Governorate to investigate the existing gap between TB-detection of the national control program SID test and that is during routine meat inspection. Additionally, to assess the cocktail antigen ELISA as a complementary test for TB diagnosis. Moreover, to estimate disease prevalence and describe the clinical signs, PM lesions, histopathological findings of bTB at the study area. Furthermore, to identify the involved mycobacteria with subsequent molecular investigation and bioinformatics analysis.

A total of 400 animals (330 cows and 70 buffaloes) were tested with SID during the national control program against bTB at Kafrelsheikh Governorate. In addition, total of 750 animals; 569 cows and 181 buffaloes in between 25 serum-sampled emergency slaughtered animals, were slaughtered at Tanta abattoir and subjected for clinical examination before slaughtering. After slaughtering, animals were subjected to PM examination.

Tuberculin-testing of 400 animals during the national control program was evaluated, many technical and procedural errors were detected, and all animals were negative. Out of them, 55 animals were tested by ELISA before the application of tuberculin test, 30 (54.5%) animals were seropositive.

A total of 25 emergency-slaughtered animals of unknown tuberculosis-status were investigated by cocktail-antigens ELISA and post-

mortem examination. Five visible tuberculous-lesion cases were detected and confirmed by PCR. ELISA was sensitive and predictive of the existence of tuberculous-lesions; 4(80%) out of 5 visible-lesion cases were seropositive. True prevalence among the slaughtered animals was 27.14%.

To confirm the effect of the reported errors on reliability of tuberculin test, reference serum of 20 tuberculosis-positive animals that were tested by standard-procedures tuberculin test and their status were confirmed by PCR after slaughtering, were tested by ELISA. Those reference samples were belonged to the TB project (No. 2966, Science & Technology Development Fund, 2017) in AHRI. A complete matching was evident, the 20 standard-tuberculin positive animals were all seropositive by the cocktail antigen ELISA.

Out of the 750 examined slaughtered animals, visible TB was detected in 4 % of animals and the TP was estimated at 6.85% (95% CI: 5.3 % – 8.9%). The disease was significantly prevalent in females than in males and there was no significant difference in prevalence between cattle and buffaloes. Lesions were of variable size contained creamy, crumbly-cheese-like, or orange pus, most (40.95%) were in lungs and its associated LNs, followed by retropharyngeal (31.8%) and mesenteric LNs (13.6%).

Mycobacterial-culture, histopathology and RT-PCR targeting all members of MBTC were performed, upon which 85%, 80% and 100% of the tested lesions of each were confirmed as TB, respectively. *Mpb70*-targetting PCR was conducted on ten of RT-PCR positive samples, the amplicons were sequenced and identified nine *M. bovis* strains and, interestingly, one *Mtb* strain from a buffalo.

Summary

Bioinformatics tools were used for the prediction of gene mutations, nucleotide polymorphisms, lineages, drug resistance and PPI of the sequenced strains. Two *M. bovis* strains were belonged to BOV AFRI lineage (Spoligotypes BOV 1; BOV 2) whilst the remaining eight strains belonged to East-Asian (Beijing) lineage that indicate their geographical origin and the role of trading in transmission of mycobacteria across countries.

The *Mtb* strain was resistant to RIF, INH and SM, and to the best of our knowledge, this is the first report of MDR-*Mtb* originating from buffaloes. In addition, seven *M. bovis* strains were resistant to EMB and ETH. INH, RIF, SM, EMB and ETH resistances were associated with *KatG*, *rpoB, rpsL, embB* and *ethA* genes mutations, respectively. Other mutations and nucleotide polymorphisms of many genes were also predicted, which either aren't associated with drug resistance in this study but previously reported as drug resistant mutations, don't confer drug resistance or other mutations which are reported for the first time and require further experimental work for their validation.

PPI analysis revealed more interactions than what would be expected for a random set of proteins of similar size and had dense interactions between nodes that are biologically connected, as a group. Moreover, the algorithms predicted additional five functional partners *(rpsG, rplB, rplP, rpsS and rpoA)* that had a biological connection with the other genes.