الملخص الإنجليزي لرسالة الدكتوراه الخاصة

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Title:

"Recent and conventional methods for identification of *Mycobacterium bovis* in farm animals"

Summary

Bovine tuberculosis is a chronic bacterial disease caused by ***M. bovis*.** It can infect many species of animals; cattle and buffaloes most commonly and are considered the maintenance hosts for the bacteria. Bovine tuberculosis can spread to humans. It is still common in developing countries, a source of economic loss, and a serious health threat to humans.

The result recorded in this study illustrated the prevalence of tuberculin reactors in dairy cattle and buffaloes in different governorates. From a total of 3600 tuberculin tested cross-bred dairy cattle including 32 herds as follows: (El-Fayoum 450 animals, Alexandria road El-Sahrawy 1850 animals, Gharbia 550 animals and Beheira 750 animals), 72 were found to be reactors with a prevalence rate of 2%. The highest herd prevalence was present in Gharbia 60% and the lowest was present El-Sahrawy, 33.3%, while it was 40% in El-Fayoum and 57.1% in Beheira. The highest result for tuberculin test was 2.7% in Gharbia and the lowest result was 1.6% in El-Sahrawy. In Beheira, it was 2.3%, and El-Fayoum, it was 2.2%.

Also, from a total of 2550 tuberculin tested buffaloes as follow (Beheira 500 animals, Gharbia 600 animals and Alexandria road El-Sahrawy 1450 animals), 26 were found to be reactors with a prevalence rate of 1%. The highest result for tuberculin test was 1.2% in Gharbia and the lowest result was 0.8% in Beheira, while in Cairo-Alexandria El-Sahrawy road, it was 1%.

The relationship between the reactivity of tested cattle and buffaloes to tuberculin test and postmortem findings was investigated in this study. In cattle, it revealed that the overall percent of PM findings with VL was 49 out of 72 (68.1%), and the percent of tuberculin reactors with NVL was 23 out of 72(31.9%). While, in buffaloes, VL was 17 out of 26 (65.4%), and the NVL was 9 out of 23 (34.6%).

Regarding the relationship between tuberculin reactors (cattle and buffaloes) and site of lesions; the 49 slaughtered tuberculin reactor cattle with VL were distributed in head 8 (11.1%), pulmonary 25 (34.7%), digestive 7 (9.7%), mixed 4 (5.6 %) and generalized 5 (6.9%). While in buffaloes, the 17 VL were distributed in pulmonary 3 (11.5%), digestive 7 (26.9%), mixed 6 (23.1 %) and generalized 1 (3.8%).

Concerning the correlation between PM finding in different age of tuberculin reactor cattle; the number of tuberculin positive reactors cattle were 12 (1.2%) from 975 animals at age from 1-3 years; 5 (41.7%) showed VL and 7 (58.3%) showed NVL. At age from 3-5 years, the number of tuberculin positive reactor cattle were 39 (2.1%) from 1900 animals; 32 (82.1%) showed VL and 7 (17.9%) showed NVL while at age over 5 years, the number of tuberculin positive reactors were 21 (2.9%) from 725; 12 (57.1%) showed VL and 9 (42.9%) showed NVL.

The total isolation rates of mycobacteria from carcasses of reactor cattle and buffaloes with and without lesions were summed up in this study. In cattle, from a total of 72 carcasses, 44 were positive cultures with an isolation rate of 61.1%, all isolates were identified as ***M. bovis***; of them, 40 (81.6%) were from VL and 4 (17.4%) were from NVL. While in buffaloes, from a total of 26 carcasses, 15 were positive cultures with an isolation rate of 57.7%; 10 isolates (38.5%) were *M .bovis*, all were from the 17 VL (58.8%), and 5 isolates (19.2%) were ***MOTT***, 3 (17.6%) were from the 17 VL and 2 (22.2%) were from the 9 NVL.

Concerning the microscopical examination by ZN staining of the 72 specimens from the slaughtered reactor cattle showed the AFB in only 26 (36.1%) samples. While in buffaloes, the 26 specimens showed the AFB in 7 only (26.9%).

On the other hand, the PM lesions (VL and NVL) of the 72 the slaughtered tuberculin reactor cattle were sorted into 4 degree of pathological lesions. Degree 0

represented all the 23 NVL (31.9%). On the other hand, the 49 VL were distributed as 3 Degree 1 (4.2%) and 23 (31.9%) for each of Degree 2 and Degree 3 which were the most predominant. Concerning the correlation between ***M. bovis*** isolation and degree of pathological lesions, out of the 72 slaughtered reactor cattle, a total of 44 ***M. bovis*** isolates were recoverd with a rate of isolation of 61.1%. Of them, 4 isolates (5.6%) were from Degree 0, 19 isolates (26.4%) were from Degree 2 and 21 isolates (29.2%) from Degree 3. No isolates were recovered from Degree 1.

Regarding the comparison between results of tuberculin test and ELISA technique using *PPD-B* and *ESAT6-CFP10* Mixture in cattle; out of 72 tuberculin reactors 49 (68.1%) serum samples were positive for ELISA using *PPD-B* and 45 (62.5%) serum samples were positive for ELISA using antigen mixture as coating antigens. And out of 49 tuberculin positive reactors with VL, 44 (89.8%) were positive for ELISA using *PPD-B* and 41(83.7%) by using antigen mixture and out of 23 tuberculin reactors with NVL, 5 (21.7%) were positive for ELISA using *PPD-B*, 4 (17.4%) were positive for ELISA using antigen mixture.

Moreover, regarding the correlation between ***M. bovis*** isolation and ELISA using *PPD-B* and antigen mixture on the sera of tuberculin reactors cattle; out of 8 tuberculin reactor cattle with head lesions, 5 (62.5%) gave ***M. bovis***isolates while 7 (87.5%) and 5 (62.5%) were ELSIA positive by using *PPD-B* and antigen mixture, respectively. Out of 22 reactors with pulmonary lesions, 22 (88%) gave ***M. bovis***isolates while 22 (88%) and 23 (92%) were ELSIA positive by using *PPD-B* and antigen mixture, respectively. Out of 7 reactors with digestive lesions, 5 (71.4%) yielded ***M. bovis***isolates, while 6 (85.7%) and 4 (57.1%) were ELSIA positive by using *PPD-B* and antigen mixture, respectively. Out of 4 reactors with mixed TB lesions, 3 (75%) gave ***M. bovis***isolates while all of them (100%) were ELSIA positive by using both *PPD-B* and antigen mixture. Out of 5 reactors with

generalized TB lesions, all of them (100%) yielded ***M. bovis***isolates and were ELSIA positive by using both *PPD-B* and antigen mixture. Out of 23 reactors with NVL, 4 (17.4%) gave ***M. bovis***isolates while 5 (21.7%) and 4 (17.4%) were ELSIA positive by using *PPD-B* and antigen mixture, respectively.

Regarding the comparison between results of tuberculin test and ELISA technique using *PPD-B* and antigen mixture in buffaloes; out of 26 tuberculin reactor buffaloes, 16 (61.5%) serum samples were positive for ELISA using *PPD-B*, while 14 (53.8%) serum samples were positive for ELISA using antigen mixture. From other side, out of 17 tuberculin positive reactors with VL, 13 (76.5%) were positive for ELISA using *PPD-B* and 12 (70.6%) by using antigen mixture. On the other hand, out of 9 tuberculin reactors with NVL, 3 (33.3%) were positive for ELISA using *PPD-B*, 2 (22.2%) were positive for ELISA using antigen mixture.

Moreover, regarding the correlation between ***M. bovis***isolation and ELISA using *PPD-B* and antigen mixture on the sera of tuberculin reactors cattle; out of 3 tuberculin reactors buffaloes with pulmonary lesions, 2 (66.7%) gave ***M. bovis***isolates and also were ELSIA positive by using both *PPD-B* and antigen mixture. Out of 7 reactors with digestive lesions, 4 (57.1%) gave ***M. bovis***isolates while 6 (85.7%) and 5 (71.4%) were ELSIA positive by using *PPD-B* and antigen mixture, respectively. Out of 6 reactors with mixed TB lesions, 3 (50%) gave ***M. bovis***isolates while 4 of them (66.7%) were ELSIA positive by using both *PPD-B* and antigen mixture. The one reactor with generalized TB lesions gave one ***M. bovis***isolate and was ELSIA positive by using both *PPD-B* and antigen mixture (100%). Out of 9 reactors with NVL, no ***M. bovis***isolates were recovered (0%) while 3 (33.3%) and 2 (22.2%) were ELSIA positive by using *PPD-B* and antigen mixture, respectively.

Regarding the sensitivity and specificity of ELISA using *PPD-B* in cattle evaluated in this study revealed 89.8% and 78.3%, respectively. On the other hand, the sensitivity and specificity of ELISA using antigen mixture revealed 83.7% and 82.6%, respectively.

Concerning the buffaloes, the sensitivity and specificity of ELISA using *PPD-B* revealed 76.5% and 66.7%, respectively. On the other hand, the sensitivity and specificity of ELISA using antigen mixture revealed 70.6% and 77.8%, respectively.

PCR test was applied on randomly selected 16 mycobacterial isolates, which are physically and biochemically identified as 14 ***M. bovis*** and 2 ***MOTT***. Of the 14 ***M. bovis*** isolates, 10 were from cattle distributed as one head, 3 pulmonary, 2 digestive, one mixed, 2 generalized forms and one NVL) and 4 from buffaloes (one from each of pulmonary, digestive, mixed and generalized forms). On the other hand, the 2 ***MOTT*** were from buffaloes (one from each of digestive and NVL). The results revealed that all the 14 isolates confirmed physically and biochemically as being ***M. bovis*** gave positive results with PCR test using Oligonucleotide primer that amplifies a 350bp fragment in *RD7* region of ***M. bovis*** (100%) and the 2 ***MOTT*** isolates gave negative results with the PCR test by using the same primer providing confirmation that it did not belong to the ***M. bovis*** as they lake the a target for primers.

Recommendations

- It is highly recommended that the application of "test and slaughter" policy is an essential mean to prevent spreading of this serious disease among cattle and buffalo herds in Egypt.
- Testing of new introduced animals at purchase to identify positive animals and avoiding purchasing old animals from markets might help decreasing within-herd prevalence.
- Good hygiene, housing and management reduce the prevalence of bovine tuberculosis.

- ELISA utilizingantigen mixture could detect early infection and also the advanced stages of infection as it counter the variability observed in antigen recognition throughout the infection process and it can be used complementary to the skin test todetermine the status of disease and reduce the frequency ofmisdiagnosis.
- The use of ELISA is suggested for situations where the investigation of the whole herd is more important than the individual testing of each animal. In addition, the ELISA can also be helpful when a collective diagnosis is desired to elucidate clinical suspicions of disease, or in the first steps of a control program, for identification of foci.
- The PCR technique can be used as a rapid confirmatory test. It is much faster than culture, reducing the time for diagnosis to 2 days and providing the ability to detect the presence of ***M. bovis*** DNA. Although direct PCR can produce a rapid result, it is recommended that culture be used in parallel to confirm the existence of a viable ***M. bovis*** in the positive reactor animals.