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Editorial

Overview on the Diagnostic Tools of Bovine Tuberculosis in Egypt

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Mycobacterium bovis is the etiologic agent of Bovine tuberculosis in cows characterized by progressive development of specific granulomatous lesions or tubercles in lung tissue, lymph nodes or other organs. Humans can be also infected through the consumption of unpasteurized milk. This route of transmission can lead to the development of extrapulmonary TB.

The diagnosis of bovine tuberculosis is based on routine testing with the intradermal tuberculin test. The Skin tests require containment of the animals on two occasions with a 72h interval for measuring the thickness of the skin fold [1].

The accuracy of the tuberculin test depends on several factors, including the use of high-quality tuberculins, the format of the tuberculin test used (e.g. single intradermal comparative tuberculin test, single intradermal tuberculin test, the caudal fold test), the efficiency of the testing procedure, the mode of interpretation of the test result as well as the immunological responsiveness of the animal at the time of the test. Furthermore, negative results (the presence of non reactors animals) does not mean that the animal is not infected or free animals which is a great important issue either for the clean non infected animals or the attendances and spreading of the infective organism in the environment..

Management is hampered in herds raised extensively on farms without adequate facilities, which is a common situation in developing countries.

This difficulty is probably one of the main reasons for why epidemiological surveys of tuberculosis planned in the eradication program are in a less advanced stage than those for brucellosis.

The results of tuberculin skin test showed low sensitivity and specificity as it revealed the animals that give positive skin test and CMI positive test should be considered highly suspect of being infected.

Such subclinical infected animals may account for the significant number of reactor cattle appearing at slaughter without lesions. Furthermore, these subclinical reactor cattle could, over time, develop disease, contributing to a significant number of herd breakdowns which are difficult to attribute to a precise source.

The Conventional culture-based detection techniques remain the golden standard technique (as mentioned by OIE (2009), so it is applied on the collected samples for detection and studying of the mycobacterium isolates even it is time consuming test and have lack In sensitivity and specificity.

The sensitivity of the cultural method was discussed by Taylor., *et al*. [2] who mentioned that the isolation of mycobacteria and their identification based on phenotypical characters. Particularly, culture methods have been constantly used as a "golden standard" but due to the fact that these methods are much more time consuming, their use is on the decline [3] and they have been replaced by other diagnostic methods.

The conventional method is currently based on acid-fast staining and cultures on solid and/or liquid media, but its sensitivity is low.

Cultures on solid media require up to 8 weeks of incubation (The times at which the *M. bovis* colonies were detected in the tubes were considerably longer to achieve the maximum Sensitivity.

As regards to isolation of *mycobacterium* species by cultural method, the viability of *M. bovis* in the organs samples is essential. The viability could be reduced by either freezing/unfreezing practices or by treatment with the selected decontaminant reagents, which is not completely innocuous for *M. bovis* and one of the main factors influencing the success of primary isolation of *M. bovis* from clinical specimens is the type of the culture media used

The low frequency at which the slants were observed (60d) could justify a certain delay for the colony detection. A factor that influences the successful mycobacterial isolation is the decontamination procedure. No ideal reagent has taking into account the sort of samples handled and the manner in which they were obtained. Not many studies exist in the literature about this matter, including the same culture media, similar samples and the same decontamination reagents [4].

Interferon gamma assay is OIE listed as an alternative test for international trade and approved as a complementary STT test as described in USDA, APHIS.

Gamma interferon assay which based on cell mediated immunity become the ideal choice in the diagnosis and express high sensitivity and specificity with specific antigens as highly sensitized PPD-B.

The IFN-γ test has been approved for use in a number of national program including in the European Union (EU), USA, New Zealand, and Australia [5] and incorporated into bovine tuberculosis eradication programs in many countries [6] either in a serial testing regime as confirmatory test after CFT to enhance specificity.

This *in vitro* assay is a laboratory based test detecting specific cell-mediated immune responses by circulating lymphocytes.

Future plan directed to use the gamma interferon assay with expected successful output. Moreover, the advantage of the IFN-γ test over the skin test is that the animals need to be captured only once.

Advantages of the IFN-c assay are its increased sensitivity, the possibility of more rapid repeat testing, no need for a second visit to the farm and more objective test procedures and interpretation in comparison to the used field test (TST). IGRAs offer the possibility of identifying BTB positive herds.

This is another useful aspect in the detection of cattle in advanced stages of tuberculosis with false-negative results on the skin test [7,8].

AS regards to PCR technique which generally applied to detect the infected samples and to confirm the isolated strains of *M. tuberculosis complex*, The standardization of the extraction method of DNA is essential because there are many extraction kits were used, most of them unsuitable for mycobacterium species .

PCR showed the highest efficiency compared to the bacteriological and microscopic examination that confirm the advantage of PCR for diagnosis of *M. tuberculosis* complex over other procedures [9]. False-positive and false-negative results, particularly in specimens containing low numbers of bacilli, have reduced the reliability of this test.

Variability in results has been attributed to the low copy number of the target sequence per bacillus combined with a low number of bacilli. Variability has also been attributed to decontamination methods, The IS6110 is not specific for *M. bovis* but is specific for all organisms in the M. tuberculosis complex, which includes *M. tuberculosis, M. bovis, M. microti* and *M. africanum* and a newly described species *M. canetti*.

However, because *M. tuberculosis* has been recovered from many animal species, infection with that organism might be more frequent in countries with a high incidence of human tuberculosis; identification of any *M. tuberculosis* complex infection would warrant a public health concern. The PCR method improves tuberculosis diagnosis, with the advantage that method is rapid and more sensitive than other methods.

PCR could be applied directly to be used as a routine diagnostic test, the evaluation of The serological tests (ELISA) which applied by using different specific antigens of mycobacteria as recommended by OIE [10], the ELISA is approved as an ancillary parallel test for farmed deer, carried out 13-33 days after the mid-cervical skin test. Alternative serum test formats have also been developed.

This test is now licensed in the USA by the USDA for some species and it is approved for use in the United Kingdom.

ELISA sensitivity has been reported to be as high as 85% by using some specific antigens (PPD and commercial mixture antigens) to improve the sensitivity and specificity of the assay [11].

Chick board titration must be carried out to standardize the dilutions of the used (antigens, serum samples and conjugate).

The ELISA technique showed variable results toward the sensitivity and specificity when compared to traditional culture technique and according to the used antigens.

The recent results of ELISA on serum samples of tuberculin tested animals showed the superiority of commercial antigens (the mixture antigens) as diagnostic antigens for bovine tuberculosis over the tradition PPD antigens. In addition, a cocktail of carefully selected antigens has the potential to be a novel diagnostic reagent.

Concerning to the some prepared antigens with STCF antigen and SONICATED antigens, the results of ELISA showed the superiority of these antigens over the tradition PPD in diagnosis of bovine tuberculosis in cattle by ELISA. On the basis of the prevalence of tuberculin skin test reactors, it suggests that a specific cocktail antigens consisting of (ESAT6 and CFP10 and other selected antigens) could be tested in the near future with large scale on field animals.

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