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THE RELATION BETWEEN THE MYCOBACTERIUM BOVIS AND PARATUBERCULOSIS IN DAIRY FARM

By

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ABSTRACT

Bovine tuberculosis (TB) caused by Mycobacterium bovis remains a major problem in a number of countries as a globally distributed zoonotic disease that poses a significant threat to livestock. Bovine tuberculosis is often found where M. avium subsp. paratuberculosis (MAP) is present and it is difficult to eradicate and needs long-term efforts especially in the presence of paratuberculosis. Paratuberculosis known as Johne's disease, it is the causative agent of the chronic enteritis disease that affect ruminants.

This study aimed to investigate TB/PTB co-infection in one dairy cattle farm and these animals had been previously skin tested. A total of 550 animals were included in this study and were examined using Tuberculin Test; 422 (76.73%) gave positive results, and then were tested by using IDEXX Enzyme Linked Immuno Sorbent Assay (ELISA) kit for screening of Bovine tuberculosis and paratuberculosis. 142 (25.82%) were positive as Mycobacterium bovis, 153 (27.82%) were positive as Mycobacterium avium subsp. paratuberculosis (Map) and 11(2.6%) were suspected as Mycobacterium avium subsp. paratuberculosis. Our results confirm the TB/PTB coinfection in these dairy cattle which 43 (7.82%) as positive T.B and Para T.B and 4 (0.72%) as positive T.B and suspected ParaT.B. Thus TB/PTB correlation was diagnosed in different dairy cattle farms. However, our information was limited. Thus, further research is needed to assess the dairy cattle status in the whole country

Keywords:

Bovine tuberculosis, Paratuberculosis, Co-infection, IDEXX ELISA test.

INTRODUCTION

Animal tuberculosis (TB) is due to infection with Mycobacterium bovis and other closely related members of the Mycobacterium tuberculosis complex (MTC). TB is relevant for public health (Mainly in developing countries) and animal health and production, since it causes severe economic losses to the livestock industry **(Allen *et al.,* 2018).**Egyptian public animal health authority recognizes single and comparative intradermal tuberculin (IDT) tests as the official bovine TB field screening tests. It is considered as a zoonotic disease that has a major risk to public health and infected man via milk consumption **(Müller *et al.,*2013).** Infection leads to a decrease in milk production (10-20%), loss of weight, reduction of fertility and condemnation of carcasses of infected animals**,** which causes severe economic losses to the livestock industry**,** and responsible for 5-10 % of all human tuberculosis cases **(Wedlock *et al.,* 2002,Collins,2006 and Gortázar *et al.,*2015)**and this happened due to that many infected animals in the herd remain undetected for a long time with shedding the bacteria by aerosol,via milk,urine and faeces.

Paratuberculosis (PTB) is a chronic infectious disease caused by Mycobacterium avium subsp paratuberculosis (Map) and is characterized by chronic granulomatous enteritis which lead to chronic diarrhea and progressive emaciation in ruminants **(González *et al.,* 2005).** Because there is no treatment or cure for Johne's disease, early detection and culling of infected cattle is critical for preventing infection in other cattle **(Hedyeh *et al.,* 2019).** The co-infection can impact both diagnostics of bTB and MAP, **(Hope *et al.,* 2005)** due to cross reactivity can impact the reaction size on the bovine injection site **(Kennedy *et al.,* 2017)** there is some epidemiological evidence that co infection can inhibit the ability to accurately identify infected animals and to clear infection from herds when the primary aim is either to eradicate bTB **(Álvarez *et al.,*2009)** or MAP **(Lilenbaum *et al.,* 2009)** Such cross-reactivity effects can lead to misdiagnosis **(Byrne *et al.,* 2018)** allowing for TB positive animals to be mistakenly retained within herds, these processes may have impacts on the ability to clear infection once detected within herds **(Hope *et al.,* 2005)** and evidence that co-infection of MAP can impact on the immunological response to tuberculin during statutory bTB skin testing **(Roupie*et al.,* 2018). Álvarez *et al.,* (2009)** suggested that co-infection of MAP and bTB can lead to problems in clearing infection**.**

MATERIAL AND METHODS

Ethical statement:

The present study was approved by institutional Animal Care and Welfare Committee Ethics (Approval No. 135/2020) Faculty of Veterinary medicine Cairo University.

Animals:

The study was performed on one cattle farm. The farm included in this study was tested with skin test. A total of 550 animals were included in the study and the blood was collected 30 days after performing IDT.

1. Tuberculin test.

All the animals were subjected to single intradermal cervical skin tuberculin test (SICST) as per the guidelines from the World Organization for Animal Health (OIE) **(Attia *et al.,* 2018).** Mammalian PPD tuberculin was prepared by Bacterial Diagnostic Products Research Department, Veterinary Serum and Vaccine Research Institute (VSVRI), Abbassia, Cairo, Egypt.

2. ELISA procedure.

The study was conducted in Animal Reproduction Research Institute for detection the presence of specific antibodies against (M. bovis) and Johne's disease (MAP) in bovine sera **(Maytham and Nidhal, 2016).** Blood samples were collected and subsequently sera separated from these samples and preserved in micro tubes and stored in aliquots at -20ºC until the ELISA test was performed **(Anita Koni1 *et al.,* 2016).**

A. Detection of specific antibodies against *Mycobacterium bovis in* Serum samples by M. bovis ELISA IDEXX Test.

The strategic supplemental use of M. bovis antibody test may increase over all diagnostic power by detecting subsets of infected animals missed by skin test **(Palmer and Waters, 2006).**

All cattle were tested in parallel with the single intradermal cervical skin tuberculin test (SICST) and the M. bovis ELISA IDEXX Test **(Anita Koni1*et al.,* 2016).**

A tuberculosis antibody-screening test was used in accordance with the manufacturer's instructions. The Mycobacterium bovis IDEXX ELISA (IDEXX M. bovis Antibody (Ab) Test.

IDEXX Europe B.V P.O. Box 1334, 2130 EK Hoofddorp, The Netherlands) **(Anita Koni1 *et al.,* 2016).**

The results of the ELISA tests were expressed as the value of the sample (S) divided by value of the positive control serum (P) supplied in the IDEXX ELISA kit, as determined by measurement of the optical density (OD) was read at absorbance of 450 nm by ELISA plate reader.3 reading obtained from each sample divided by the value of the positive control was used to calculate the S/P value for each sample **(Anita Koni1 *et al.*, 2016).**

$$S/P = \frac{\text{Sample A (450)} - \text{NCX}}{\text{PCX} - \text{NCX}}$$

S/P=sample to positive ratio

SA= absorbance of sample

NCX=mean negative control

PCX= mean positive control

The criteria used to determine the status of animals tested by ELISA were as follows: An S/P value <0.3 was judged to be negative; an S/P value equal to or greater than 0.3 was considered positive**. (Anita Koni1 *et al.*, 2016).**

B. Detection of specific antibodies against Mycobacterium avium subsp. paratuberculosis in Serum samples by (MAP) ELISA IDEXX Test.

A Paratuberculosis antibody-screening test was used in accordance with the manufacturer's instructions. [[The Mycobacterium Paratuberculosis IDEXX ELISA (IDEXX Paratuberculosis Antibody (Ab) Test. IDEXX Europe B.V P.O. Box 1334, 2130 EK Hoofddorp, the Netherlands) **(Anita Koni1*et al.*, 2016).** The result is obtained by comparing the sample Optical Density with the positive control mean optical density**. (Gormley *et al.*, 2006).**

$$S/P\ \% = 100\text{x}\frac{\text{Sample A (450)} - \text{NC A(450)}}{\text{PCX} - \text{NC A (450)}}$$

S/P%= sample to positive ratio percent

SA= absorbance of sample

NCA= absorbance negative control

PCX= mean positive control

The criteria used to determine the status of animals tested by ELISA were as follows: an S/P% value equal to or lower than 45% was judged to be negative; an S/P% value equal to or greater than 55% was judged to be positive and if S/P% value greater than 45% and lower than 55% was considered suspect . **(Anita Koni1*et al.,* 2016).**

RESULTS

From the inspection of the 550 Holstein cows in this farm by IDT, The number of positive animals was 422 (76.72%) and the negative animals were 128 (23.28%) as shown in (Tables 1, 2). ELISA TEST RESULTS for the positive IDT animals showed that 116 (27.48%) from the 422 animals are positive by M. bovis -ELISA, 87 (20.6%) positive by JD-ELISA and 11 (2.6%) suspected to Johne's disease.

Table (1): Results of skin tuberculin Test in examined cows.

Total cows	Positive to skin tuberculin test (%)	Negative to skin tuberculin test (%)
550	**422 (76.72%)**	**128 (23.28%)**

Table (2): Elisa test results for m. bovis and (map) for cows positive to tuberculin test.

Total cows positive	Positive to M. bovis (%)	Positive to JD (%)	Suspected to JD (%)
422	**116 (27.48%)**	**87 (20.6%)**	**11 (2.6%)**

In (Table 3), the examination of the negative animals for IDT (128) by ELISA test showed that 26 (20.31%) positive for M.BOVIS and 66 (51.56%) were positive for Johne's disease.

Table (3): Elisa test results for M .bovis and (map) for cows negative to tuberculin test.

Total cows negative	Positive to M. bovis (%)	Positive to JD (%)
128	26 (20.31%)	66 (51.56%)

Chart 1 show the percentage of infection in the farm and it was 26% positive T.B, 28% positive Para T.B, 2% suspected Para T.B and the percentage of negative cows in the herd was 44%. In (Table 4) the results showed that, the mixed infection in the farm between T.B and Para T.B were 42 animals (7.82%) and the animals were positive T.B and suspected positive Para T.B were 4 animals (0.72%) and this may lead that there relation between T.B and Para T.B infection.

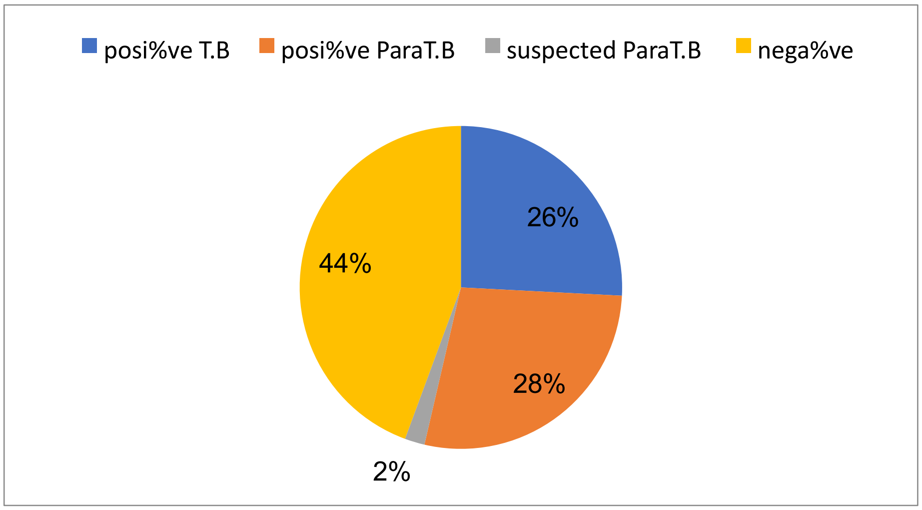

Chart (1): infection distribution inside the farm.

Table (4): The relation between mixed infection of T.B and Para T.B.

Total animals	**Positive T.B And Para T.B**		**Positive T.B And suspected Phar.B.**	
	NO	**%**	**NO**	**%**
550	**43**	**7.82%**	**4**	**0.72%**

DISCUSSION

The consolidation of the dairy industry, with increase in sizes, density and productivity of the herds, was associated to unprecedented bovine tuberculosis (bTB) prevalence levels in dairy herds. Where Johne's disease (JD), another mycobacterial disease, is also prevalent. Here, we aimed to characterize the association between bTB- and JD-diagnostic results in two heavily bTB- and JD- confected dairy herds. **Picasso-Rissoa *et al.,* 2019.**

Bovine tuberculosis (bTB) is a worldwide-distributed chronic infectious disease of cattle caused mainly by infection with Mycobacterium bovis (M. bovis), which represents a threat to animal and public health. Bovine tuberculosis is a zoonotic disease affecting cattle worldwide, causing economic losses due to eradication programs and trade limitations **(Pollock and Neill, 2002**). In spite of great efforts the Officially Tuberculosis Free status has not yet been achieved in some countries throughout Europe. This lack of success has been attributed, among other causes, to the insufficient sensitivity of the diagnostic tests under certain field conditions (**Morrison *et al.,* 2000).** Mycobacterial infections, both bovine tuberculosis (BTB) and mycobacteriosis, continue to threaten the health of both animals and humans. One of the most widespread nontuberculous mycobacterial (NTM) infections is paratuberculosis, also known as Johne's disease. Its etiological agent, Mycobacterium avium spp. paratuberculosis (MAP), causes granulomatous enteritis. Paratuberculosis (paraTB) mainly occurs in ruminants and may cause significant economic losses for breeders **(Whittington *et al.,* 2019).**

As the diagnosis of mycobacterial infections, both those caused by Mycobacterium tuberculosis complex (MTBC) and NTM, still presents difficulties, there is a need to improve diagnostic tests. One such difficulty is presented by the influence of cross-reactive immune responses towards different mycobacteria. Many studies have found MAP infection to impact BTB test results, while others report no significant effect **Didkowska *et al.,* 2021**. It is also known that exposure to environmental NTM can reduce the specificity of M. bovis diagnostic tests. However, only a few studies have examined the inverse relationship: the effect of MTBC infection on MAP diagnostic results. The aim of this study was to determine the effect of M. bovis infection on the results of serological testing for paraTB in cows, and to compare the accuracy of the tuberculin skin test (TST), the occurrence of gross lesions, and ELISA testing for diagnosing MTBC in cattle**.** In spite of investing great efforts in the fight against tuberculosis, achievement of complete eradication has been inconsistent in certain regions or countries due to wildlife reservoirs that disseminate the infection via infected animal's movement **(Morrison *et al.,* 2000, Pollock and Neill, 2002 and Corner, 2006)**. Other major factors hampering eradication programs are limitations of routine diagnostic tests (mainly skin tests), in particular concerning their sensitivity. Diagnostic accuracy is a key issue in the test-and-slaughter programs applied in Spain and many other countries, especially at the present low-prevalence stage, when detection of all infected animals is crucial **(Pollock *et al.,* 2001).**

The office International des Epizooties (OIE) considers these diseases of major global importance and categorizes these diseases as list B transmissible disease. OIE also considered it of socioeconomic and public health importance within countries and is significant for the trade of animals and their products **(OIE manual 2008).**

Intradermal tuberculin test is the most used diagnostic tests in the world with serological tests which can be an ancillary diagnosis for bovine tuberculosis **(Soares Filho *et al.,* 2020).** As showed in (Table, 1) for a total number of 550 tested cows using skin test in one herd. The results revealed that 422 (76.72%) cow's positive to skin tuberculin test and 128 (23.28%) negative to skin tuberculin test.

Co-infection affected the sensitivity of the skin test **(Roupie *et al.,* 2018)**. Other researchers have found during experimental co-infections that the performance of the skin test (based on Purified Protein Derivative (PPD) tuberculin injection) can be affected **(Barry *et al.,* 2011).** If MAP decreases the specificity of the comparative tuberculin test (CTT), then more false positives would be disclosed. This could increase MAP disclosure rates (i.e., could result in uninfected herds being classed as experiencing a breakdown **(Nuñez-Garcia *et al.,* 2018),** in these conditions, the ELISA test should improve the possibility of finding chronically infected animals. So several new ELISA kits become available used as diagnostic technology is often based on serum antibody detection using enzyme-linked immunosorbent assay (ELISA) technology because of its low cost and high-throughput potential through automation **(Michael *et al.,* 2005).**The sensitivity of ELISA tests is generally high in animals with clinical disease. The Mycobacterium bovis IDEXX ELISA Test has the advantage of being a more rapid test and it is less vulnerable to errors and biases on the farm. Also **Anita Koni *et al.,* (2016) and (Maytham and Nidhal, 2016)** confirmed that absorbed ELISA test could be used as valuable diagnostic tool in detection of bovine Paratuberculosis in cattle herds with a previous history of infection with Johne's Disease and considered as economic test to screen the herds and to eliminate any positive case**.**

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