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SERUM LYSOZYME, IL-1 AND IL-6 PROFILES IN MICE EXPERIMENTALLY INFECTED WITH *MYCOPLASMA BOVIS*

By

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ABSTRACT

Mycoplasma bovis infection is an economically important disease in dairy and feedlot cattle. The pathogen is a common bacterium found in mucous membranes in animal species, including respiratory, urogenital, and gastrointestinal tracts. *M. bovis* infection is a highly contagious disease with over 70% infection rate. The prevalence of *M. bovis* infection is wide and highly reported among young calves. The mortality is usually reported in 2-6 weeks old calves while the peak clinical incidence is at the age of one month. In adult dairy cows, the organism takes a long period (months to years) to colonize the mammary gland. The aim of the present study was to measure interleukins 1 & 6 and lysozyme as parameters of the host immune response to *M. bovis* in mice experimentally infected with the pathogen. The expirement was carried out on 20 albino mice (6-weeks-old) divided into two equal groups. The experimental group was interprotineally (I/P) inoculated with 1ml containing 1x 10^7 CFU of fresh prepared *M. bovis* while the control group received phosphate buffered saline (PBS). Animals were bled from the tail vein under the Laboratory Animal License by collecting approximately 30 µl of blood into clean capillary tubes at, 1st, 3^{r}d and 7th day post infection. Serum samples were collected for measurements of lysozyme, IL-1 and IL-6 levels using ELISA kits. The study denoted general activation of immune response in the infected mice with markedly increased lysozyme, as well as interleukins 1 and 6. Strong immune response was detected at the 7th day post infection as revealed by elevated Il-1 & 6 levels. From the obtained results, it could be concluded that the cell-mediated immunity seems to play an important role in an elimination of *M. bovis* from the host and *M. bovis* infection effectively stimulated the cell-mediated immunity in mice..

Keywords:

Mycoplasma bovis; Lysozyme; mice; Interleukins-1 & 6.

INTRODUCTION

Infection of dairy and feed cattle by mycoplasma is a global issue with consequences for cattle health and economics **(Askar *et al.*, 2021**). *M. bovis* is responsible for bronchopneumonia, pharyngitis, laryngitis, otitis, keratoconjunctivitis, meningitis, and mastitis, as well as metritis, infertility, and abortion in all ages. The bacterium can be shed and survive for many years in contaminated herds **(Dudek *et al*. 2016).** *M. bovis* infection has substantial economic implications for dairy and beef industries as it may result in calf mortality, weight loss and significant drop in milk production **(Hertl *et al.* 2014).**

M. bovis has been isolated from milk, conjunctiva, semen, and vaginal secretions. Airborne transmission is the main route of infection in the susceptible bovine host. Poor hygienic practices in dairy farms are the major factors in occurrence and transmission of *M. bovis* mastitis **(Fox, 2012 and Fox *et al.*, 2008)** or through the purchase of sub-clinically infected carrier non-lactating animals **(Nicholas *et al.*, 2016).** ELISA test has been optimized for field samples of both serum and milk. Prevention of *M. bovis* infection in cattle is usually carried out by sanitary control measures, antimicrobial therapy and vaccination **(Priyantha *et al.*, 2021)**. The use of antibiotics against mycoplasma infection is often ineffective and cause a global problem such as tetracycline- and spectinomycin-resistant bacteria **(Kroemer *et al.*, 2012).**

M. bovis prevents lymphocyte proliferation due to its immune-suppressive characteristics and promotes lymphocytic apoptosis in response to mitogens **(Mulongo, *et al.*, 2014).** Once gets into the host, *M. bovis* attaches cell surfaces and migrates intracellularly into neutrophils and macrophages; that occurs through phagocytosis. Some studies indicated that *M. bovis* induces activation and development of different cytokines from macrophages, as IFN-γ, interleukin-4, TNF-α, and nitric oxide **(Van der Merw *et al*., 2010)**.

The immune response is divided into humoral immunity and cellular immunity, with cellular immunity regulated by T-cells and B-cells regulating humoral immunity. The immune system develops cytokines including interleukins, which balance humoral and cell-mediated immune responses **(Srikumaran *et al.* 2007**). On the other hand, innate immune defenses are comprised of physical barriers as surfactant proteins, lysozyme, and antimicrobial peptides **(Houlihan**

***et al*. 2007**).

Cytokine profiles elicited by microorganisms and their products play a key role in disease expression in their natural hosts **(Mu *et al.*, 2000).**

The cellular immune response is more successful than the humoral response in the elimination of *M. bovis* from the host **(Dudek *et al.*, 2020).** T-cells differentiate into four sub-types, based on the micro environmental cytokines involving IL-1, IL-4, IL-5, IL-6, IL-10, IFNs, I12, IL-17, IL-23, and TNF-α, and TGF-β. Th1 cells activate macrophages by releasing IFN-γ. Th2 cells activate B-cells by synthesizing cytokines and antibodies involved in the pathogen neutralization, opsonization, phagocytosis and immune regulation. Regulatory T-cells control the immune responses by developing the cytokines IL-10 and TGF-β **(Maunsell and Chase, 2019**).

Although the mechanisms of *M. bovis* pathogenesis require more investigation, a broad range of immunomodulatory events have been exerted by the pathogen. Such modulation affects directly and indirectly leukocytes by the induction of cytokine secretion. In the lung of calves experimentally infected with M. bovis, immune cell activation resulted in expression of cytokines such as interleukin (IL)-4, IL-10 and interferon (IFN)-γ and tumor necrosis factor (TNF)-α capable of inducing lung lesions and hyperplasia of the bronchus-associated lymphoid tissues **(Ohtsuka *et al.*, 2020)**.

IL-1 has been found to be important in inflammatory responses as it stimulates T cells and B cells and activates macrophages and natural killer cells. IL-1 is a self-inducer IL-1 and induces production of other inflammatory mediators such as TNF, IL-8, IL-6, nitric oxide, and prostaglandin-E by monocytes **(Jennifer *et al.*, 2003).**

On the other hand, circulating IL-6 levels are normally very low or undetectable and are markedly increased in several diseases associated with inflammation, inducing the transition from acute to a chronic inflammatory response. IL-6 is a multi-functional cytokine that drives terminal B cell differentiation and secretion of immunoglobulins. IL-6 also cooperates with IL-21 to promote differentiation of CD4+ T follicular helper cells **(Pelosi *et al.*, 2015).**

The aim of the present study was to estimate the levels of interleukin 1, interleukin 6 and lysozyme as host immune response parameters to *M. bovis* infection in experimentally infected mice.

MATERIAL AND METHODS

Animals:

The study was carried out on 20 albino mice (6 weeks old). After adaptation for a week, the mice were divided into two groups; experimental and control ((10 each).

***Mycoplasma bovis* inoculum:**

The bacterial strain used in the study was *Mycoplasma bovis* was kindly supplied from Animal Reproduction Research Institute, Giza, Egypt. The bacterial inoculum was prepared from freshly grown batch suspended in PBS with a density of 1 x $\mathbf{10^7}$ bacterial CFU/ml **(Tian and Ba, 2012).**

Mouse inoculation:

Each mouse of the experimental group was inoculated intrapretoneally with 0.5 ml of the prepared *M. bovis* inoculum while the control ones received phosphate buffered saline (PBS) instead. Mice of both groups were housed individually in ventilated wire cages at temperature of 23 ± 3°C and humidity 50–70 %. Feed and water were offered to mice of both groups *ad libitum*. The experiment was performed according to the guidelines of the Institutional Animal Care and Use Committee of Cairo University, Egypt.

Serum samples:

Approximately, 200 µl blood was taken from the tail vein for isolation of about 60 µl serum **(Stenina *et al.*, 2012)**. Blood was collected from each animal into a plain clean Ependorf tube at 1st, 3rd and 7th day post infection. Serum was separated by centrifugation at 2000-3000 RPM for 10 minutes. Serum samples were stored at -20℃ for further usage.

Lysozyme concentration:

Lysozyme concentration in serum of control and experimental mice was measured 3 days post inoculation with *M. bovis*. A commercial kit was used for determination of the lysozyme level in each sample (Mouse Lysozyme ELISA Kit, Kementech Company, Denmark) following the supplier instructions.

IL-1 and IL-6 levels:

Determination of the IL-1 and IL-6 concentrations in serum samples of mice was carried out using the ELISA kits supplied by MABTECH, SWEDISH BIOTECH. The concentrations were reported in pictograms per milliliter (pg/ml) following the kits supplier instructions.

Statistics:

The data were processed statistically using an online program **(calculator *et al.*, 2020)**.

RESULTS AND DISCUSSION

Lysozyme concentration, expressed in µg/ml serum of mice infected with *M. bovis* (no= 10) as compared to non-infected mice (no. = 10), is depicted in table 1. Significant elevations were observed at the individual and the mean levels at the third day post infection.

Table (1): Lysozyme concentrations in *M. bovis*-infected at three days post inoculation.

Groups	Individual levels										Mean ± *SD*
	1	2	3	4	5	6	7	8	9	10	
Control	**7**	**6.5**	**7.8**	**6.6**	**7.6**	**8.1**	**7.9**	**6.8**	**7.1**	**6.4**	7.18±0.59
Test	**22**	**31**	**21**	**23**	**29**	**32**	**28**	**37**	**26**	**35**	28.4±5.18

- **Lysozyme concentration = µg/ml**

Table (2) shows that, the concentration (pg/ml) of IL-2 was significantly increased in the *M. bovis*-infected mice with a significant gradual elevation at the 1, 3 and 7 days post infection. Meanwhile, the IL-6 level (pg/ml) showed a profile similar to that of IL-1 (Table 3) Fig. (1, 2).

Table (2): Level of IL-1 in *M. bovis*-infected mice at 1, 3 and 7 days post inoculation.

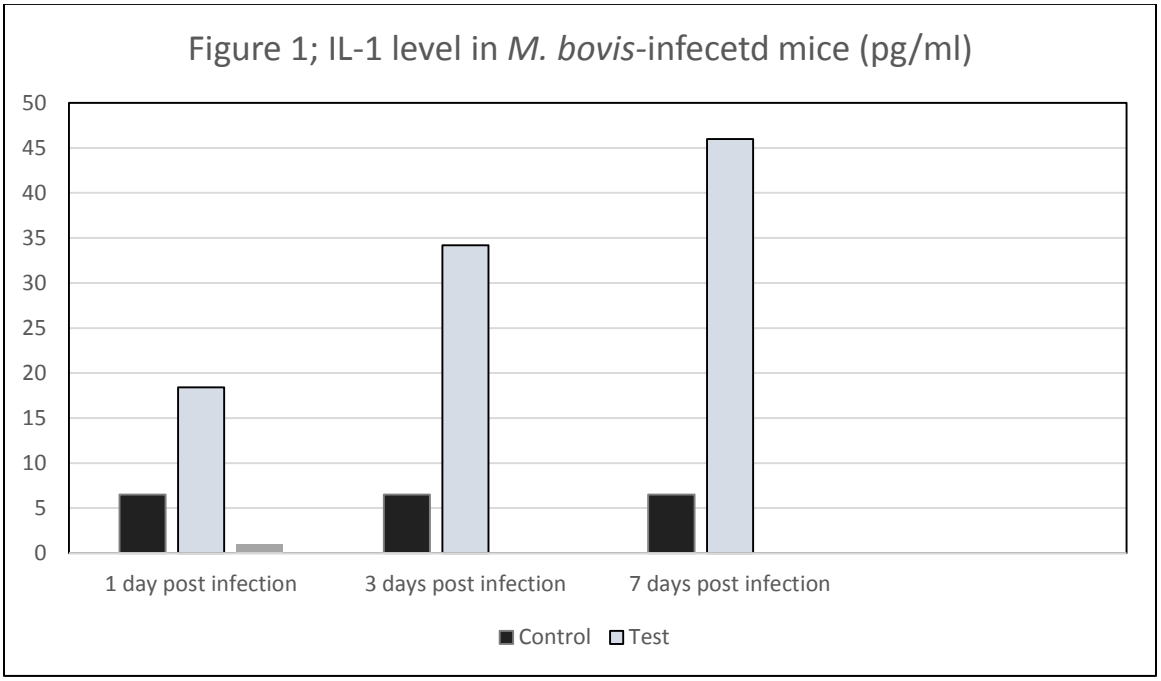
Groups	Individual values										Mean ± *SD*
	1	2	3	4	5	6	7	8	9	10	
Control	**9***	**7**	**2**	**0.8**	**12**	**0.6**	**10**	**0.5**	**11**	**12**	**6.49 ±4.72**
Test: day 1	**15***	**17**	**15**	**21**	**16**	**24**	**18**	**18**	**17**	**23**	**18.4±3.03**
Test: day 3	**32***	**29**	**31**	**39**	**38**	**28**	**39**	**41**	**27**	**38**	**34.2±5.03**
Test: day 7	**33***	**42**	**39**	**49**	**44**	**50**	**55**	**48**	**43**	**57**	**46±6.91**

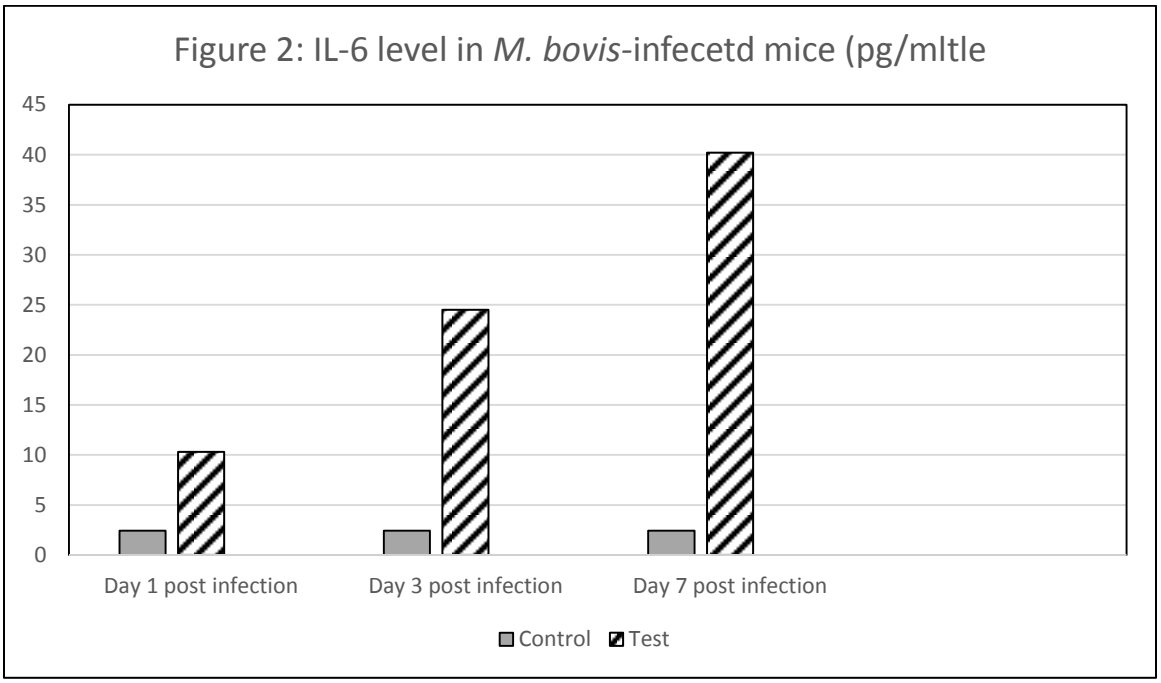
- **IL-1 = pg/ml**

Table (3): Level of IL-6 in *M. bovis*-infected mice at 1, 3 and 7 days post inoculation.

Groups	Individual values										Mean ± *SD*
	1	2	3	4	5	6	7	8	9	10	
Control	**2***	**0.9**	**1**	**2**	**5**	**3**	**0.8**	**4**	**5**	**0.6**	**2.43±1.63**
Test: day 1	**9***	**11**	**8**	**13**	**7**	**12**	**11**	**10**	**13**	**9**	**10.3±1.95**
Test: day 3	**22***	**19**	**27**	**26**	**31**	**16**	**20**	**24**	**28**	**32**	**24.5±4.98**
Test: day 7	**41***	**39**	**35**	**42**	**44**	**47**	**39**	**43**	**36**	**36**	**40.2±3.7**

- **IL-6= pg/ml**





The obtained results indicate activation of lysozyme, IL-1 and IL-6 synthesis levels in mice with *M. bovis* as evidenced by the markedly increased concentration of lysozyme and both interleukins. Similar results were demonstrated in previous studies where a positive correlation were recorded between the increased ELISA titers of IL-1 and IL-6 levels of mice in response to *M. bovis* infection **(Nicholas *et al.*, 2002; Dudek *et al.*, 2016).**

Circulating IL-6 levels are normally low and are markedly increased in several diseases associated with inflammation, inducing the transition from an acute to a chronic inflammatory response **(Pelosi *et al.*, 2015).** This could be the case of mycoplasmosis, as a chronic disease. *Mycoplasma* spp. was found to enhance innate immune cells to produce pro-inflammatory cytokines such as IL-1, IL-6 as well as chemokines **(Vanden and Rosenbusch, 2003).**

Results of the current study showed that strong immune response of mice following inoculation with *M. bovis* detected at the 7th day post infection as indicated by elevated of IL-1 and IL-6 levels. A finding that was recorded by **Prysliak *et al*. (2013).**

It has been reported that *M. bovis* can induce the synthesis and production of important cytokines (TNF-α, IL-1 β, IL-1α, IL-2, IL-12, IFN-γ and IL- 6) in the infected host **(Gondaira *et al.* 2015).** A finding which means that *M. bovis* infection results in increased levels of the IL-1 and IL-6 proinflammatory cytokines that could be essential essential for

M. bovis pathogenesis **(Hermeyer *et al.* 2012; Valsala *et al.* 2017**). A finding that is supported by the notification that *M. bovis* infects and modifies the cellular machinery of host monocytes to prolong their survival and facilitate subsequent systemic distribution **(Mulongo *et al.*, 2014).**

Although changes of serum T cell cytokines such as IL-4 and IFN-γ were observed in experimental intra-tracheal infection of M. bovis of calves, the relationship between the outcome and T cell cytokines levels in clinical cases of mycoplasma infection is not known **(Ohtsuka *et al.*, 2020).**

In conclusion, *Mycoplasma bovis* can cause a wide variety of immune response by activating cytokine secretion from lymphocytes, macrophages, and neutrophils. Therefore,the cell-mediated immunity seems to play an important role in an elimination of *M. bovis* from the host. However, further studies of the host immune responses to *M. bovis* infection are required for better control and prevention of the disease.

Conflict of interest:

The author declares that there is no conflict of interest.

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