



## Responses of inflammatory cytokines in non-pregnant Boer does inoculated with *Corynebacterium pseudotuberculosis* via various routes

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### Abstract

*Corynebacterium pseudotuberculosis* is the causative agent of caseous lymphadenitis in sheep and goats and is characterized by the development of pyogranulomas in the lymph nodes and organs. This study was designed to measure the concentration of inflammatory cytokines (IL-1 $\beta$  & IL-6) in experimental non-pregnant Boer does inoculated with *Corynebacterium pseudotuberculosis* through various routes. Little is known about the concentration of IL-1 $\beta$  and IL-6 in different routes of infection caused by the organism. A total of twenty healthy non-pregnant Boer does were divided into 4 groups (A-D) of 5 does per groups. Three groups (A-C) were inoculated with 10<sup>7</sup>cfu/1ml of live *Corynebacterium pseudotuberculosis* through intradermal, intranasal and oral routes respectively, while group D was kept unexposed. Following infection, blood samples were collected from the jugular vein between three days interval period for the analysis of IL-1 $\beta$  and IL-6. A significant increase (P<0.05) in IL-1 $\beta$  was observed through intranasal, intradermal and oral route compared to the control group. Significant increase in IL-1 $\beta$  and IL-6 was observed through intranasal route compared to other routes. This study, therefore, highlights the effects of inflammatory cytokines (IL-1 $\beta$  and IL-6) in caseous lymphadenitis infection.

**Keywords:** Caseous lymphadenitis; *C. pseudotuberculosis*; non-gravid Boer does; interleukin-1 $\beta$ ; Interleukin-6

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### Introduction

Caseous lymphadenitis, commonly called CLA is a chronic contagious infectious disease of sheep and goats (Dorella et al., 2006; Fontain and Baird, 2008), and can also infect several other hosts, including human (Dorella, et al., 2006; Baird et al., 2007; Guimaraes et al., 2011). The etiological agent is *Corynebacterium pseudotuberculosis*, a facultative intracellular anaerobic is a small rod curve bacterium (Jesse, 2011). The disease is distributed worldwide and has a major economic impact in most sheep and goats (Baird and Fontaine, 2007; Seyffert et al., 2010; Guimaraes, et al., 2011). *Corynebacterium pseudotuberculosis* can be transmitted through various ways. Some experimental

infection of *C. pseudotuberculosis* with different routes of administrations such as intraperitoneal, intravenous, intranasal, intratracheal, intravaginal, oral and intradermal have also been reported (Goldberger et al., 1981; Brown and Olander et al., 1987; Fontaine et al., 2006; Adza Rina et al., 2013). Interleukin-1 $\beta$  (IL-3) is a potent mediator in response to infection and injury (Dinarello, 1998) and interleukin-6 (IL-6) subfamily is a group of hematopoietic cytokines with a broad range of physiological functions including cell survival, immune and inflammatory responses (Jazayeri et al., 2010). Reichlin et al. (1993), Chrousos (1995), Ehrhart et al. (1996) and Gonzalez et al. (1994, 1995 & 1996) stated that increase in IL-1, IL-6 and tumour necrosis factor (TNF) influences the activities between the

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immune system and hypothalamic pituitary adrenocortical (HPA) axis. Progesterone and estradiol are stimulated by IL-1 in small follicles while in antral gonadotropin dependent follicles and secretion are inhibited (Baratta et al., 1996). Mikuni (1995) and Kylie et al. (1998) found that IL-6 has no effect on progesterone but inhibits estradiol production.

There is paucity of knowledge on the responses of IL-1 $\beta$  and IL-6 in non-pregnant Boer does during caseous lymphadenitis CLA infection. Therefore, this study was designed to describe the response of IL-1 $\beta$  and IL-6 during *C. pseudotuberculosis* infection.

## Materials and Methods

### Ethical consideration

The experimental procedure was conducted under the approval of the Animal Care and Use Ethics Committee, University Putra Malaysia as required in Malaysia by the Animal welfare Act (2014) with reference No. (UPM/IACUC/AUP-R29/2014).

### Animals and management

Twenty adult healthy non-pregnant Boer does, with average weight of 30 $\pm$ 5kg were used in this study. The animals were acclimatized for 2 weeks prior to the experiment and were fed with commercial goat pellets (300g/goats/day) with cut Napier grass. Blood from the jugular vein and swab samples from the nasal, vagina and oral mucosa were collected for screening of *C. pseudotuberculosis* before the commencement of the experiment. The does were randomly divided into four groups (A, B, C and D) equal groups of five animals per group.

### Preparation of inoculum

*C. pseudotuberculosis* was isolated from an outbreak of clinical CLA cases among goats at University Putra Malaysia was used in this study. The bacterium was inoculated into brain heart infusion (BHI) broth and incubated at 37°C for 48h. Plate count method of Alcamo (1998) was used to determine the bacteria concentration.

### Inoculation

The animals in group A was inoculated with 10<sup>7</sup> colony forming unit/ml of live *C. pseudotuberculosis* through intradermal route at the neck region. Animals in the experimental group B was inoculated with the same dose and inoculated through intranasal route while group C was given the same inoculum orally. Lastly those in group D was given 1 ml of phosphate buffer saline (PBS) pH 7.0 orally and served as the control. Following infection, the animals were observed daily for clinical signs for 30 days.

### Blood sampling

The blood samples were collected from the jugular vein from the control and infected goats twice weekly throughout the duration of experiment, using a 1.2 x 38 mm (21 G 1.5") Venoject needle (Precision Glide™, Becton Dickinson, UK) with Venoject holder (Vacutainer®, BD vacutainer™, USA) into 5 ml plain tubes (Vacutainer®, BD vacutainer™, USA). The sera were extracted and kept at -20°C for IL-1 $\beta$  and IL-6 analysis.

### IL-1 $\beta$ and IL-6 analysis

IL-1 $\beta$  concentration in serum was measured using commercial goat interleukin 1 $\beta$  (IL-1 $\beta$  Elisa kit (CUSABIO®), Lot-V09117007 and CSB-E14360G and IL-6 concentration in serum was measured using commercial goat interleukin 6 (IL-6) Elisa kit (CUSABIO®), Lot-029117008 and CSB-E14360G. All procedures were performed according to manufacturer's instructions. The samples were performed in duplicate including the control and standard and all steps were carried out at room temperature. Absorbance of the reaction was read at 450 nm (Sunrise Tecan Austria, GmbH) and the absorbance of each sample was then calculated. The log of concentrations (IL-1 $\beta$  and IL-6) was plotted against the log of O.D and the best fitted lines were determined by regression analysis.

### Statistical analysis

Data were analysed using statistical software JMP (version 9.0.1 SAS Institute Inc., Cary, NC, USA). Two-way analysis of variance (ANOVA) was used to test the differences between specific pairs. The differences were considered as significant when P<0.05.

## Results

The results of IL-1 $\beta$  and IL-6 in non-pregnant Boer does experimentally inoculated with *Corynebacterium pseudotuberculosis* are given in Table 1. The results showed that serum IL-1 $\beta$  and IL-6 was significantly high (P<0.05) in intranasal inoculation compared to control and other routes.

## Discussion

The present study showed a significant increase in IL-1 $\beta$  in all the groups following infection with *C. pseudotuberculosis* compared to the control group. The increase in the concentration of IL-1 $\beta$  may contribute to the increased of progesterone hormone in non-pregnant does that may lead to infertility. This result is supported by the earlier findings of Verhoeven et al. (1988) who reported that both isoform of IL-1 $\alpha$  and IL-1 $\beta$  stimulate basal progesterone secretion. Similarly, Chen et al.

**Table 1: Mean and Standard deviation of interleukin 1 $\beta$  and interleukin 6 responses in non-pregnant Boer does experimentally inoculated with *Corynebacterium pseudotuberculosis***

Group	IL-1 $\beta$ pg/ml	IL-6pg/ml
Intranasal	98.0 $\pm$ 29.91 <sup>a</sup>	82.60 $\pm$ 72.71 <sup>a</sup>
Intradermal	67 $\pm$ 35.66 <sup>b</sup>	16.7 $\pm$ 19.49 <sup>b</sup>
Oral	42 $\pm$ 36.10 <sup>c</sup>	6.35 $\pm$ 2.72 <sup>b</sup>
Control	4 $\pm$ 2.95 <sup>d</sup>	4 $\pm$ 2.25 <sup>b</sup>

<sup>a,b,c,d</sup>different superscripts in a row differ significantly (P<0.05).

(2000) also reported that IL-1 $\beta$  stimulates progesterone production by human granulosa and theca cells. The increased in IL-1 $\beta$  showed that infection by the bacteria has taken place. This finding is also supported by Zychlinsky et al. (1994) who reported that huge quantities of IL-1 $\alpha$  precursor and mature IL-1 are being released prior to time of macrophages apoptosis.

From our experiment, the most important and striking observation is that IL-6 increased through intranasal route thirty days post treatment. The precise mechanism by which IL-6 increased only through this route has not yet been established. However, our results for the first time, reported that IL-6 significantly increase via the intranasal route in the early stage of *C. pseudotuberculosis* infection *in vivo*. Jones et al. (2001) and Kishimoto et al. (1995) indicated in their studies that IL-6 blood levels are elevated in numerous infectious, inflammatory, and autoimmune diseases and in cancer in association with increased synthesis of other cytokines stimulated by infection, trauma, and immunological challenge. The rise of IL-6 in the present study suggest that IL-6 level corresponds with the severity of the disease. The current study is in agreement with the outcome of Kaplanski et al. (2003) who stated that IL-6 is the main cytokine involved in the change of acute to chronic inflammation. it is also involved in the development of specific cellular, humoral immune responses, including end-stage B cell differentiation, immunoglobulin secretion and T cell activation. The elevation of IL-6 through the intranasal route observed in this study may be as useful biomarker since the concentration had a very statistical significance in the experimental animals and this mechanism could be very important in the vaccine development and control of CLA infection in farm animals.

In conclusion, *C. pseudotuberculosis* infection through the intranasal route can significantly affect the IL-1 $\beta$  and IL-6 concentrations, which alternately affect the reproductive performance of does. This may also be useful in the diagnosis and control of caseous lymphadenitis in early stage of infection.

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