CLINICAL RESPONSE AND PATHOLOGICAL CHANGES ASSOCIATED WITH *CORYNEBACTERIUM PSEUDOTUBERCULOSIS* INFECTION IN MICE

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Abstract

Corynebacterium pseudotuberculosis is a causative agent of caseous lymphadenitis (CLA). Caseous lymphadenitis is a chronic granulomatous infectious disease characterised by the formation of abscesses, typically located in superficial lymph nodes and lungs. Difficulties in early clinical identification of CLA-infected animals have limited the effectiveness of controlling and eradicating this disease. This study was conducted to acquire a better understanding the pathogenesis of CLA through the mice model. Sixteen healthy male mice were divided equally into 2 groups, where the first group of mice were orally inoculated with 1.0 mL of sterile phosphate buffer solution (PBS), pH 7 and the second group of mice were orally inoculated with 1.0 mL of 10^9 colony forming unit (CFU) of *C. pseudotuberculosis*. Clinical signs and histopathological changes in visceral organs were compared between the diseased and non-diseased group within the period of 120 hours of post-inoculation. Data was analyzed using the SPSS version 19. This study showed that there were significant ($p<0.05$) differences in histopathological changes in the lungs, liver and kidneys between diseased and non-diseased mice. Clinical signs were not observed.

Keywords: *Corynebacterium pseudotuberculosis*, caseous lymphadenitis (CLA), mice, clinical response, cellular changes

INTRODUCTION

Caseous lymphadenitis (CLA) or cheesy gland is a disease that can potentially threaten the livestock industry of Malaysia. This disease is found in the major sheep and goat production areas worldwide, causing significant economic losses (Paton *et al*., 2003; Williamson, 2001; Stoops *et al*., 1984). The causal pathogen of CLA is *Corynebacterium pseudotuberculosis*, a rod shape, gram-positive, non-spore forming bacteria. It is a facultative intracellular parasite that is found on fomites, in soil and manure contaminated with prudent exudate (Cahn and Line, 2005). Caseous lymphadenitis is a chronic granulomatous infectious disease of sheep and goats characterised by the

formation of abscesses, typically located in superficial lymph nodes and lungs (Batey, 1986; Paton *et al*., 1994; Arsenaut *et al*., 2003).
Transmission of CLA among sheep or goats occurs mainly through contamination of superficial wounds that commonly appear following routine procedures, such as shearing, castration and ear-tagging, or body injuries caused by traumatic events (Dorella *et al*., 2006). Controlling CLA with antimicrobial or antibiotics is challenging because the bacteria stay protected inside abscesses due to the thick capsule that surrounds them (Piontkowski and Shivvers, 1998; Williamson, 2001). In general, the best strategy to control this disease is by vaccination of healthy animals and identification as well as removal of the infected animals (Menzies *et al*, 2004; Paton *et al*., 1995; Williamson, 2001). However, the difficulties in early clinical identification of infected animals have caused a limitation to the effectiveness of this strategy. A number of serodiagnostic tests have been developed to overcome the problem of clinical identification of CLA in infected animals. Most tests have been reported to lack either sensitivity or specificity (Burrell, 1980; Menzies and Muckle, 1989; Menzies *et al*, 2004; Williamson, 2001). Therefore, this study was conducted to determine a better method of detecting and diagnosing CLA in subclinical cases through the mice model. Furthermore, there is a lack of information on the pathogenesis and clinical signs of CLA in mice infected experimentally via oral route infection. Thus the objective of the present study was also to determine the development of clinical signs and pathological changes in mice following the oral route inoculation with *C. pseudotuberculosis*.

MATERIALS AND METHOD

Experimental animals
Four-week old healthy mice (16 ICR, Institute of Cancer Research) were used in this study. The mice were kept at a stocking density 8 per cage in an air-conditioned room. They were fed commercial mice pellets and drinking water *ad libitum* throughout the study. The mice were acclimatised for a period of 1 week before the commencement of study.

Bacteria
Blood bacterial agar culture made from a lymph node that was naturally infected with caseous lymphadenitis from a previous CLA outbreak at the Taman Pertanian Universiti, Universiti Putra Malaysia and was culturally and biochemically identified as *C. pseudotuberculosis*. The bacteria was then subcultured in Brain Heart Infusion broth for 24 hours and concentration estimated to the standard dose of 1×10^9 CFU/mL using the MacFarland technique.

Experimental design
The mice were separated into Group 1 (non-diseased) and Group 2 (diseased) with 8 mice per group. Each mouse in Group 1 was orally inoculated with 1.0 mL of sterile phosphate buffer saline (PBS), pH 7. The mice in Group 2 were orally inoculated with 1.0 mL of 10^9 CFU of *C. pseudotuberculosis*. Development of clinical signs and mortality were observed in the mice within 120 hours after inoculation. Immediate post-mortem examination was performed on mice that died within and after 120 hours of

post-inoculation. Heart, lungs, lymph node, kidney, intestine and brain tissue samples were obtained for histopathology. All changes and abnormalities were recorded.

Clinical signs and scoring

The clinical signs recorded were ruffled hair, eye discharge, movement and responsiveness. Clinical signs scoring for ruffled fur in mice was evaluated and scored according to the criteria in Table 1.

Table 1. Clinical signs and lesion scoring in mice treated with *C. pseudotuberculosis*

Criteria	Score			
	0	1 (Mild)	2 (Moderate)	3 (Severe)
Clinical Sign				
Ruffled fur	Normal fur	30% of body	60% of body	>60% of body
Movement	Normal movement & appetite	30% reduction	60% reduction	>60% reduction
Eye discharge	Normal, no discharge	30% discharge	60% discharge	>60% discharge
Responsiveness	Normal	30% reduction	60% reduction	>60% reduction
Lesion	Normal	<30% of field	30-60% of field	>60% of field

Lesion scoring done on heart, lung, lymph node, liver, kidney and intestine tissues fixed in 10% formalin and brain tissue fixed in 40% formalin and stained with haematoxylin and eosin. Examination and scoring under 200 × magnification.

Histopathology method and lesion scoring

Post-mortem examination was conducted on all mice that died within 120 hours after inoculation. Mice that survived after 120 hours were euthanised by cervical dislocation and post-mortem performed. Heart, lung, lymph nodes, liver, kidney, intestine and brain tissues were obtained for histopathology evaluation. These tissue samples were fixed with 10% formalin except for the brain tissue which was fixed with 40% formalin and processed in an automatic tissue processor. The tissues were processed in paraffin blocks and stained with haematoxylin and eosion. Cellular changes were scored following observation of 5 slides per organ. Six microscopic fields for each slide were observed under 200× magnification. Lesion scoring is according to Table 1.

RESULTS AND DISCUSSION

Clinical signs

Within 120 hours after inoculation of *C. pseudotuberculosis*, the mice did not show clinical signs. These findings are not similar to that of previous studies where significant clinical signs were observed in mice induced to develop the disease (Jesse *et al*. 2011). However in some cases, as in sheep and goats, CLA infections do produce clinical signs (Brown et al., 1987). It is possible that the short observation period (5 days) of the study

and the effectiveness of different inoculation routes may have contributed to the variation in observations. It is possible that had the mice had been observed longer, the disease might have developed to show clinical signs and progress to other organs (Gorman *et al*., 2010). However, post-mortem examination is still necessary to confirm diagnosis.

Histopathological findings

There was no significant histopathological change in the non-diseased group of mice but the diseased mice did show significant ($p<0.05$) changes in lungs, liver and kidneys. The lungs showed infiltration of neutrophils and macrophages, congestion, increased vascularization, thrombosis, hemorrhages in the alveolar lumen bronchiole and formation of microabscesses. In the liver of diseased mice there were neutrophils and macrophages infiltrations, degeneration, vacuolation (necrosis), haemorrhage and formation of microabscesses, while the kidneys showed hemorrhages, degeneration, congestion and tubular necrosis.

The lesions observed in this study were similar to those found in sheep and goats infected with CLA via other routes of inoculations (Brown *et al*., 1986) and in mice (Jesse *et al*., 2011). The lesions in the lungs, liver and kidneys may be due to two main virulence factors of *C. pseudotuberculosis*, namely phospholipase D (PLD) and mycolic acid (Hodgson *et al*., 1992). Phospholipase D is a potent exotoxin of *C. pseudotuberculosis* that promotes hydrolysis of ester bonds in mammalian cell membrane, resulting in the damage or destruction of host cell membranes leading to dysfunction or disruption (Salyers and Witt, 1994), which is lethal at high doses to different laboratory and domestic animals (Songer, 1997). Mycolic acid on the other hand causes pyogranulomatous lesions, localized swelling, congestion and necrosis in the lymph nodes, liver, kidneys and mammary glands (Jolly, 1966; Valli and Parry, 1993). The spread of infection in the host may occur via the blood or lymphatic system (Williamson, 2011).

CONCLUSION AND RECOMMENDATION

This study shows that oral route inoculation of *C. pseudotuberculosis* can produce CLA in mice. Future researches that include ultrastructural studies need to be conducted on larger scale and for longer periods to determine obtain more conclusive results. The knowledge and understanding gained from these further studies will be able to contribute to the development of an effective treatment, control and eradication programs for the disease.

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