

## Pathological Changes in the Organs of Mice Model Inoculated with *Corynebacterium pseudotuberculosis* Organism

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### ABSTRACT

*Corynebacterium pseudotuberculosis* is a facultative, gram positive intracellular small club-shaped rod which produces lesions similar to those of tuberculosis. It is known worldwide to cause caseous lymphadenitis (CLA) in sheep and goats. CLA is characterized by the abscess formation in lymph nodes and/or visceral organs. In the recent outbreak of CLA in the TPU farm in UPM, it was reported that the CLA lesions were found in the visceral organs. In the present investigation, attempts were made to study the ethiopathogenesis of CLA in mice models which involved a comparison of clinical signs, haemogram and biochemistry, and histopathological changes in visceral organs between the diseased and non-diseased group. As an overall summary of this project, CLA in mice resulted in clinical signs, such as huddling together, dejection, anorexia, pasty faeces and accompanied by a rapid and shallow respiration pattern. For the haemogram and serum biochemistry profile, it showed a significant difference in the mean value between the diseased group and non-diseased group which include lymphocyte, plasma protein, monocyte, eosinophil, total bilirubin, total protein and potassium. Finally, the most pronounced histopathological changes in the visceral organs were septicaemia, with severe congestion and increased vascularization, together with the presence of capsulated abscess, micro-abscesses formation, infiltration of neutrophils and macrophages, tubercle granulomas, necrosis and early signs of degeneration in majority of the infected mice.

**Keywords:** *Corynebacterium pseudotuberculosis*, caseous lymphadenitis (CLA), ethiopathogenesis, septicaemia, tubercle granuloma

### INTRODUCTION

In Malaysia, the small ruminant population has been steadily increasing over the past ten years. According to the livestock statistics provided by the Ministry of Agriculture, the total population of sheep and goats in Malaysia was 469,620 heads in 2008 (<http://www.moa.gov.my>). However, this livestock industry is plagued by diseases, which are the main causes of morbidity and mortality, reducing productivity and incurring losses for farmers. One such

important disease is Caseous Lymphadenitis (CLA), which is caused by *Corynebacterium pseudotuberculosis*.

*Pseudotuberculosis* (formerly known as *c. ovis*) is a facultative, gram positive intracellular small club-shaped rod which produces lesions similar to those of tuberculosis. It is known worldwide to have caused CLA in sheep and goats. *C. pseudotuberculosis* is a very hardy organism that survives well in the environment and can infect a variety of animal species,

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including humans. CLA is characterized by the abscess formation in lymph nodes and/or visceral organs. The affected sheep or goats typically have abscesses in the parotid or retropharyngeal lymph nodes, and the disease can be diagnosed through bacteriological culture of pus from such abscesses. However, a proportion of the infected sheep or goats may have only internal abscesses, often in the lungs or mediastinal lymph nodes and show no overt clinical signs of infection. The recent outbreak of CLA at the TPU farm in UPM reported that the CLA lesions were also found in the visceral organs (Jesse *et al.*, 2008). The disease is distributed worldwide, with cases being reported in Europe, Australia, North and South America, Africa and the Middle East (Dorella *et al.*, 2006). CLA causes economic losses for pedigree sheep/goat breeders and concerns have been raised that the disease may spread to commercial and small holder flocks and lead to a decrease in quality and increased condemnation of carcass at slaughter. In countries with large numbers of sheep, such as Australia, the disease causes considerable financial losses through condemnation and down-grading of affected carcasses at meat inspection (Paton, 1990). Nonetheless, information on the pathogenesis and clinical signs in mice model is scarce.

Therefore, in the present investigation, attempts were done to study the ethiopathogenesis of CLA in mice models which involve comparing the clinical signs, haemogram, and serum biochemistry profile, as well as histopathological changes in visceral organs between the diseased and non-diseased groups.

## MATERIALS AND METHODS

### *Animals*

120 apparently healthy mice, about 7 weeks of age, were used in this study. They were kept in the stocking density of 10 mice/cage in an air conditioned room, fed with commercial mice pellets and drinking water, which were freely available for an acclimatization period of 1 week before the beginning of the study.

### *Bacteria*

Blood agar culture made from a lymph node that was naturally infected with caseous lymphadenitis was previously culturally and biochemically identified as *C. pseudotuberculosis*. This was sub-cultured in Brain Heart Infusion (BHI) broth for 24h and concentration were estimated to the standard dose of  $1 \times 10^9$  CFU/ml using the Mac Farland technique.

### *Experimental Design*

The experiment was carried out in two separate sets (Set A and Set B), in order to study the different objectives. In both the sets, the mice were divided into 2 groups. The diseased group comprised of 30 mice, each of which was injected intraperitoneally with 0.2 ml (approx.  $1 \times 10^9$  CFU/ml) of the infective inoculums. Meanwhile, the non-diseased group also comprised of 30 mice, served as the control group which was not infected. After the inoculation, clinical signs were observed in both the experimental mice sets. For Set A, 15 post-inoculation, the blood samples were collected and processed for haematology and biochemistry profile. In Set B, an immediate post-mortem examination was performed on mice which died throughout the experiment.

### *Histopathology*

The collected organs were placed in 10% buffered formalin. Paraffin-embedded sections were then routinely stained with haematoxylin-eosin (H&E).

## RESULTS AND DISCUSSION

The pictures show the histopathological changes that took place after the *Corynebacterium pseudotuberculosis* organism inoculated after 15 hours.

Meanwhile, the histopathological changes in the diseased group of mice were generally those of septicaemia, in which there were generalised congestion of the organs in all the infected mice. The changes were most

pronounced in the liver and kidney, whereby the presence of tuberculous granuloma (caseating tubercule), giant multinucleated cells, infiltration of neutrophils and macrophages, degeneration, vacuolation (necrosis), haemorrhage, and formation of microabscesses, were detected. For the lungs, the most pronounced lesion was congestion and increased vascularisation, whereas for the heart, besides the common finding of congestion, there was one sample from the infected mice that showed the formation of the abscess with signs of calcification and infiltration of macrophages.

The histological findings from the visceral organs of the infected mice in this study are

similar to the histopathological changes in the *C. pseudotuberculosis* infected sheep. In addition, there was a formation of caseous abscesses found in the sample of liver and the development of abscesses present in multiple visceral organs. Therefore, the pathogenesis of the lesion could be explained using the sample from the study carried out on the sheep. In CLA, once a lymph node has been colonized by *C. pseudotuberculosis*, it will undergo a short period of generalized inflammation. Phospholipase D, the soluble exotoxin produced by *C. pseudotuberculosis*, is the probable initiator of this lymphadenitis. Pepin *et al.* (1991) reported that within 24h of the subcutaneous inoculation of lambs, a

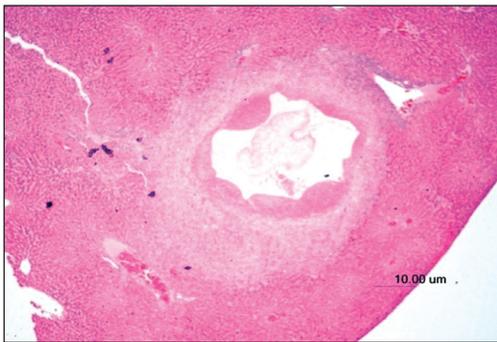


Fig. 1: Liver. Presence of capsulated abscess. Several distinct concentric layers are discernible within the lesion. Several distinct concentric layers are discernible within the lesion. Centrally, there is liquefactive necrosis (liquid pus; A). (HE, x40)

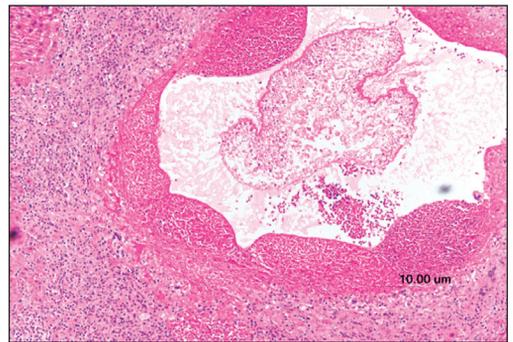


Fig. 2: Liver. Showing the border between the layers of caseous necrosis and active immature fibrosis containing mononuclear inflammatory cells (C). A thick layer of mature fibrosis (B) delineates the extent of the lesion (HE, x100)

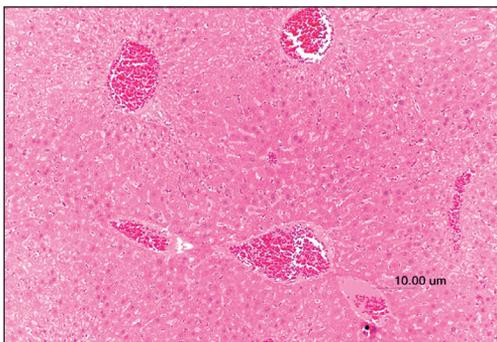


Fig. 3: Liver. Markedly congested (A) and vacuolation indicating necrosis (B) (HE, x40)

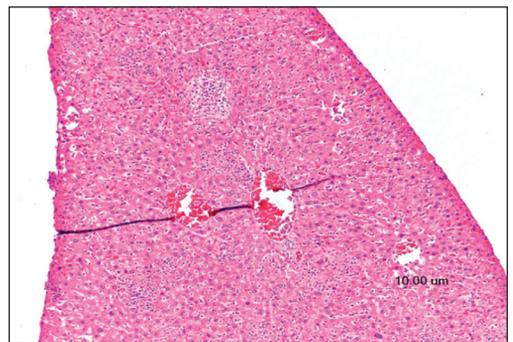
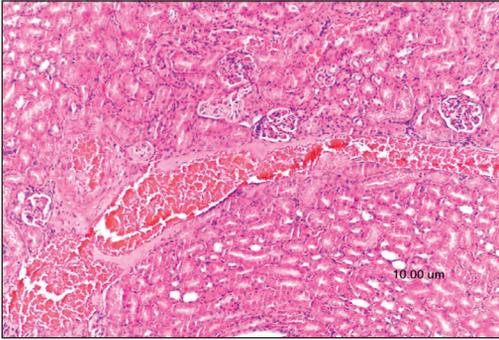
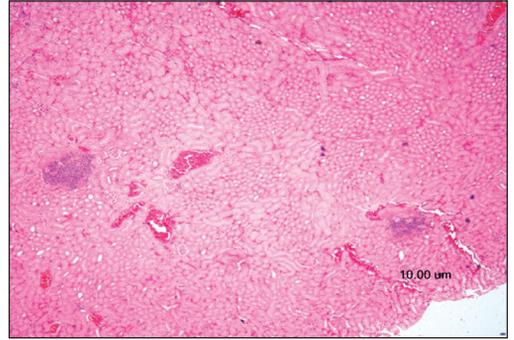


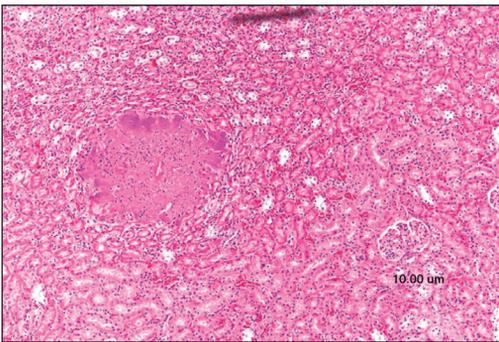
Fig.4: Liver. Micro abscesses formation (A); congestion (B) (HE, x40)



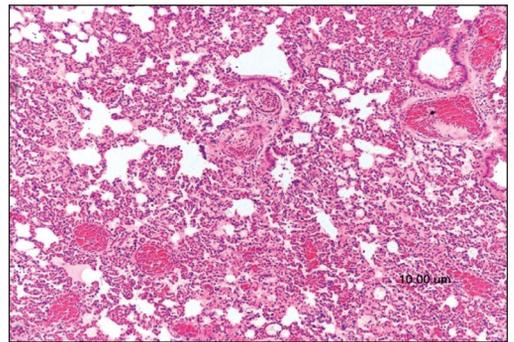
*Fig. 5: Kidney. Congestion (A), intertubular hemorrhage (B) and signs of early degeneration (C). (HE, x40)*



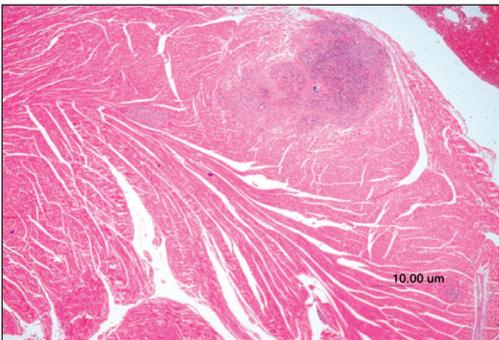
*Fig. 6: Kidney. Severe intertubular hemorrhage (A) and early stage of abscess formation (B) (HE, x40)*



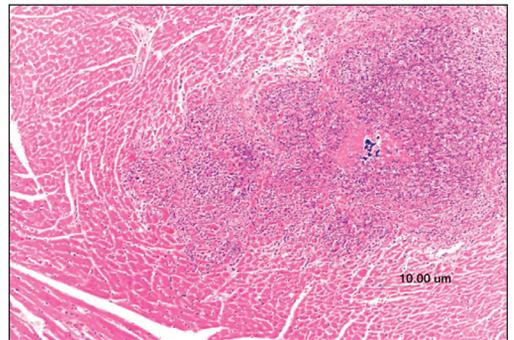
*Fig. 7: Kidney. Severe interlobular hemorrhage (A) and tuberculous granuloma (B) (HE, x40)*



*Fig. 8: Lung. Severe congestion (A) and massive influx of inflammatory cells (HE, x40)*



*Fig. 9: Heart. Abscess formation with sign of calcification (HE, x40)*



*Fig. 10: Heart. Presence of macrophages infiltration and neutrophils (HE, x100)*

number of micro abscesses were observed within the cortical region of the lymph node draining the site of inoculation. By day six of post-inoculation, these micro abscesses had become more numerous and began to expand, with coalesce forming larger purulent foci. The early pyogranulomas contained clumps of bacteria and cellular debris. At the same time, and in parallel with the cellular events at the point of entry, the infiltration of neutrophils diminished and monocytes or macrophages became the predominant cell type within the lesion (Pepin *et al.*, 1994). A process during which the lesion was encapsulated followed shortly thereafter, leading to a diminution of the inflammatory reaction in the parenchyma of the node. In the early stages, the purulent contents of the abscess were soft and semi-fluid; as time progressed, however, the pus within the lesion took on a more plastic or solid form, in which scattered clumps of bacteria were sometimes noted. Meanwhile, the most pronounced histopathological changes in the visceral organs were septicemia with severe congestion and increased vascularization, together with the presence of capsulated abscess, micro-abscesses formation, infiltration of neutrophils and macrophages, tubercule granulomas, necrosis, and early signs of degeneration in majority of the infected mice.

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