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Clinico-Pathological Changes in Mice Following Experimental Infection with Whole Cell and Exotoxin (PLD) Extracted from *C. pseudotuberculosis*

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Abstract: The purpose of the study was to describe the systematic infections of caseous lymphadenitis in mice with special reference to clinical manifestation and pathogenesis. In this, 104 apparently healthy mice, 2-3 weeks of old were selected and divided into 3 groups, namely control, whole cell and exotoxin groups. Mice of whole groups were exposed intraperitoneally to 1 mL of the inoculums containing 10^9 Colony-Forming Unit (CFU)/mL of live *C. pseudotuberculosis*. Exotoxin group were infected intraperitoneally with a single dose of exotoxin extracted from *C. pseudotuberculosis*. Control group were exposed intraperitoneally to 1 mL of phosphate-buffered saline. Groups of exotoxin and whole cell challenged showed prominent clinical signs that were characteristic of CLA which included depression, anorexia, submandibular oedema, yellowish and bloody diarrhea, ruffled caot, eye discharges, poor general condition and occasionally partial tremor of hindquarters. In necropsy, visceral abscess was the condition recorded in experimentally infected mice. There were also congestion and hemorrhages in the lungs, liver, spleen, kidney and intestine. Microscopically, there were lesions in the form of tuberculous granuloma (caseating tubercule), presence of giant cells, infiltration of neutrophils and macrophages, degeneration, vacuolation (necrosis), hemorrhage and formation of microabscesses. The results of this study indicated that manipulation of the organism and its extracted exotoxin (PLD) in experimenatal animals (mice model) led to clinical signs and pathological alterations that were similar to those conducted on small ruminant research of experimentally nature.

Key words: Caseous lymphadenitis, *C. pseudotuberculosis*, PLD, experimental indection, mice

INTRODUCTION

Corynebacterium pseudotuberculosis has long been recognized as the etiological agent of Caseous Lymphadenitis (CLA), a chronic suppurative necrotizing inflammation of lymph nodes in small ruminants (Pekelder, 2000; Baird and Fontaine, 2007; Robles, 2007). This organism is also the causative agent of other chronic infection in cattle and horses; these include ulcerative lymphangitis in cattle and horses and external and internal abscesses in horses (Aleman *et al*., 1996; Spier and Whitcomb, 2007). Infections due to *C. pseudotuberculosis* has also been reported in buffalos, camelids, equids and rarely in humans (Peel *et al*., 1997; Selim, 2001; Anderson *et al*., 2004; Braga *et al*., 2006; Jain-Lambert *et al*., 2006). Phospholipase D (PLD) has been identified as a potent exotoxin in *C. pseudotuberculosis* and a key virulence factor in the development of CLA. Studies have shown that this exotoxin (PLD) is necessary for establishment of the disease (Hodgson *et al*., 1992, 1994, 1999; McNamara *et al*., 1994; Simmons *et al*., 1998). Despite the worldwide significance of caseous lymphadenitis in small animal industry, little information exists concerning the clinical and systemic effects of *C. pseudotuberculosis* and its exotoxin, a sphingomyelin-specific phospholipase D (Soucek *et al*., 1971). Therefore, this study of experimental nature reports for the first time observations in mice experimentally infected with *C. pseudotuberculosis* and its exotoxin (PLD). As it will serve to elucidate this disease in mice with objective of establishing a base line data in order to further develop a preventive method of control against caseous lymphadenitis in small ruminants.

MATERIALS AND METHODS

Animals: About 104 apparently healthy mice, 2-3 weeks of old were used in this study. Animals were maintained under the same condition where they were kept in the stocking density of 5 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.

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Inoculum preparation (*C. pseudtuberculosis*): Blood agar culture made from a lymph node that was naturally infected with caseous lymphadenitis was earlier culturally and biochemically identified as *C. pseudotuberculosis*. These isolated organisms were revived by subculturing onto newly prepared blood agar 24 h at 37°C. The cultures were then harvested and suspended in normal saline solution where concentration estimated to the standard dose of 1×10^9 CFU mL^{-1} using the Mac Farland technique.

Inoculum preparation of exotoxin (PLD): About 250 mL of frozen toxin which was earlier extracted from the organism (*C. pseudotuberculosis*) as described above were thawed prior inoculation.

Experimental design: The experiment was carried out in three separate groups, namely control whole bacterium and exotoxin groups in order to study the different objectives. Exoxin and whole bacterium group contained 40 mice each while the control contains 24 mice only. Each animal in whole bacterium group was injected intraperitoneally with 1 mL (approximately 1×10^9 CFU mL^{-1}) of the infective inoculum (*C. pseudotuberculosis*) while in exotoxin group, each animal was intraperitoneally inoculated with 1 mL of exotoxin earlier extracted from *C. pseudotuberculosis*. Similarly, the infection was carried out in control group where each animal was challenged intraperitoneally with 1 mL of PBS. After the inoculation, clinical signs were observed at least twice daily through the trial and mortality rate recorded in all groups involved in the study. Ten animals from exotoxin and whole bacterium groups and 3 animals from control group were humanely sacrificed by cervical dislocation at every sampling date (12, 24, 48, 72, 96, 120, 144 and 168 h post inoculation). Just prior the sacrificing, blood samples were collected for hematology and biochemistry profile by cardiac puncture using a 26G ×1.5 Venoject needle (PrecisionGlide™, Becton Dickinson, UK) with venoject holder (Vacutainer®, BD vacutainer™, USA). The sampled animals were then immediately submitted for postmortem examination. During necropsy, gross lesions were observed and vital organs such as liver, spleen, heart, lung, intestine, abdominal lymph nodes were collected for histopathological examination.

Clinical scoring: Briefly, the clinical signs of all the three groups namely, control, whole bacterium and exotoxin were scored in scale of 0-3 based on the presence of inappetance (food and water intake), rough coat, ability to move and discharges from the eye as well as the degree of eye discharges following infection of *C. pseudotuberculosis* and its exotoxin (PLD). The score 0 represented no abnormality of clinical signs observed, 1 for mild (20% abnormal), 2 for moderate (20-51% abnormal) and 3 for severe (51-70% abnormal).

Histopathology: Samples of the liver, spleen, kidneys, heart, lung, intestine, abdominal lymph nodes were collected and placed in 10% buffered formalin. Similarly, brain was also collected but fixed in 40% buffered formalin. The samples were trimmed into the required orientation with thickness of 0.5 cm and processed by dehydration in series of alcohol solution of different concentration using an automated tissue (Leica TP 1020, Germany). The samples were then embedded Paraffin (Reichert-Jung Paraffin Embedding Center), trimmed at 5 μm and sectioned at 4 μm with microtome (Leica 2045, Germany). The paraffin sections were then transferred into the water bath (Lieca Hi 1210, Germany) and mounted on glass slides (Sail Brand 7101, China). Paraffin-embedded sections were then routinely stained with Haematoxilin and Eosin (HE) dye. Histopathological changes were recorded by examining the slides under magnification of 20x using light microscope.

Statistical analysis: The statistical package SPSS Software 17 (PASW Version 17; Inc., Chicago, IL, USA) was used. Clinical values were subjected to Kruskal-Wallis test while values of histopatholgical results were summarized and subjected to Analysis of Variance (ANOVA). Treatment and time were included as fixed factors. An error level of 0.05 was used.

RESULTS AND DISCUSSION

Clinical observation: All challenged groups were severely affected following experimentally infection of whole bacterium and exotoxin extracted of *C. pseudotuberculosis*.

In whole bacterium group, the clinical signs due to the infection appeared at day 2 post infection (pi) where most of the animals (mice) exhibited typical clinical signs which included anorexia, severe depression, ruffled coat, eye discharges and yellowish diarrhea which moderately increased after 48 h post-infection (Fig. 1). The mean rank of food-water intake, fur and depression was significantly ($p<0.05$) higher in *C. pseudotuberculosis* group than control and exotoxin group at day 2-6 post challenged (Table 1-3, respectively). No significant difference ($p<0.05$) was observed in eye discharges between this group and control group (Table 4).

Table 1: Means rank of food and water intake through experimental period between those infected with *C. pesudotuberculosis* and its exotoxin (PLD) and control group

	Groups		
Days	Control	*C. pseudotuberculosis*	Exotoxin (PLD)
0	$1.5^{a,x}$	$1.5^{a,x}$	$3.0^{b,z}$
1	$1.5^{a,x}$	$1.5^{a,x}$	$3.0^{b,z}$
2	$1.0^{a,x}$	$3.0^{b,y}$	$2.0^{c,y}$
3	$1.0^{a,x}$	$3.0^{b,y}$	$2.0^{c,y}$
4	$1.0^{a,x}$	$3.0^{b,y}$	$2.0^{c,y}$
5	$1.5^{a,x}$	$3.0^{b,y}$	$1.5^{a,x}$
6	$1.5^{a,x}$	$2.0^{a,x}$	$1.5^{a,x}$
7	$2.0^{a,x}$	$2.0^{a,x}$	$2.0^{a,x}$

Table 2: Mean rank of movement (depression) through experimental period between those infected with *C. pseudotuberculosis* and its exotoxin (PLD) and control group

	Groups		
Days	Control	*C. pseudotuberculosis*	Exotoxin (PLD)
0	$1.5^{a,x}$	$1.5^{a,x}$	$3.0^{b,z}$
1	$1.5^{a,x}$	$1.5^{a,x}$	$3.0^{b,z}$
2	$1.0^{a,x}$	$3.0^{b,y}$	$2.0^{c,y}$
3	$1.0^{a,x}$	$3.0^{b,y}$	$2.0^{c,y}$
4	$1.0^{a,x}$	$3.0^{b,y}$	$2.0^{c,y}$
5	$1.5^{a,x}$	$3.0^{b,y}$	$1.5^{a,x}$
6	$1.5^{a,x}$	$2.0^{a,x}$	$1.5^{a,x}$
7	$2.0^{a,x}$	$2.0^{a,x}$	$2.0^{a,x}$

Table 3: Means rank of fur through experimental period between those infected with *C. pesudotuberculosis* and its exotoxin (PLD) and control group

	Groups		
Days	Control	*C. pseudotuberculosis*	Exotoxin (PLD)
0	$1.5^{a,x}$	$1.5^{a,x}$	$3.0^{b,z}$
1	$1.0^{a,x}$	$2.5^{b,y}$	$2.5^{b,z}$
2	$1.0^{a,x}$	$3.0^{b,z}$	$2.0^{c,y}$
3	$1.0^{a,x}$	$3.0^{b,z}$	$2.0^{c,y}$
4	$1.0^{a,x}$	$3.0^{b,z}$	$2.0^{c,y}$
5	$1.5^{a,x}$	$3.0^{b,z}$	$1.5^{a,x}$
6	$1.5^{a,x}$	$2.0^{a,x}$	$1.5^{a,x}$
7	$2.0^{a,x}$	$2.0^{a,x}$	$2.0^{a,x}$

Table 4: Means rank of eye discharge between those infected with *C. pesudotuberculosis* and its exotoxin (PLD) and control group

	Groups		
Days	Control	*C. pseudotuberculosis*	Exoxin (PLD)
0	$1.5^{a,x}$	$1.5^{a,x}$	$3.0^{b,z}$
1	$1.5^{a,x}$	$1.5^{a,x}$	$3.0^{b,z}$
2	$1.5^{a,x}$	$1.5^{a,x}$	$3.0^{b,z}$
3	$1.5^{a,x}$	$1.5^{a,x}$	$3.0^{b,z}$
4	$1.5^{a,x}$	$1.5^{a,x}$	$3.0^{b,z}$
5	$2.0^{a,x}$	$2.0^{a,x}$	$2.0^{a,x}$
6	$2.0^{a,x}$	$2.0^{a,x}$	$2.0^{a,x}$
7	$2.0^{a,x}$	$2.0^{a,x}$	$2.0^{a,x}$

$^{a-c}$Means with different superscripts with in row differed significantly ($p<0.05$) due to treatment effect. $^{x-z}$Means with different superscripts with in column differed significantly ($p<0.05$) due to treatment effect

In exotoxin group, the clinical course was characterized by severe depression, ruffled coat, rigorous inappettance, bloody diarrhea, swelling in cranio cervical region and bilateral eye secretions (Fig. 2-4). Most of these clinical signs were observed between 6-30 h post-infection. The mean rank of food-water intake, movement (depression) and eye discharges in exotoxin group at day 1 was significantly ($p<0.05$) different when compared to *C. pseudotuberculosis* and control group (Table 1-4). Mean rank of fur in exotoxin group was

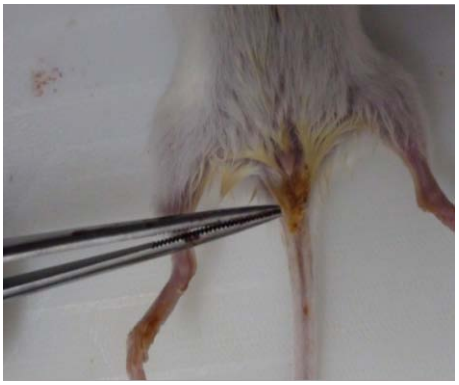

Fig. 1: Yellowish diarrhea in mouse at day 3 post *C. pseudotuberculosis* challenged

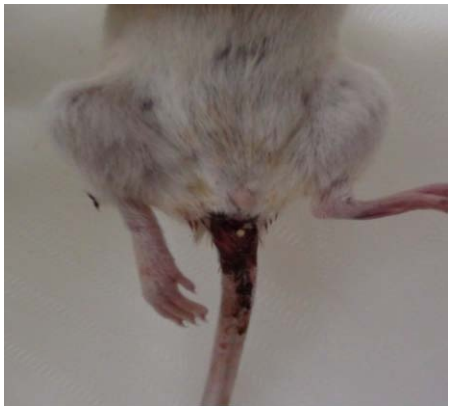

Fig. 2: Bloody diarrhea in a mouse post at day 2 post exotoxin (PLD) challenged

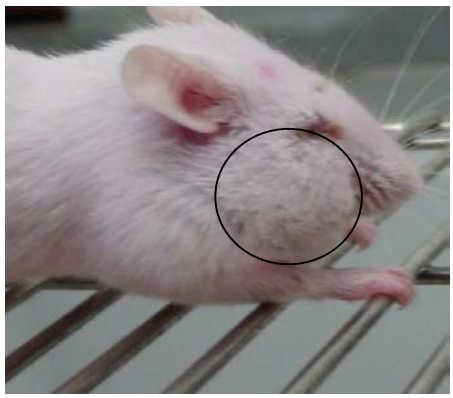

Fig. 3: Swelling in mouse at day 2 post challenged with exotoxin (circle)

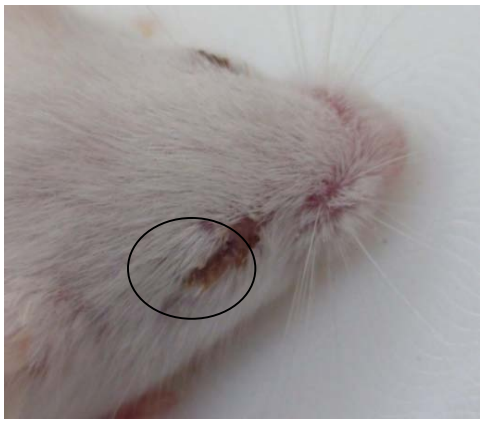

Fig. 4: Discharges from the eye in a mouse at day 2 postchallenged with exotoxin (circle)

Table 5: Involvement of different visceral organs in mice challenged with *C. pseudotuberculosis* and its exotoxin (PLD)

	Groups		
Parameters	Control	*C. pseudotuberculosis*	Toxin (PLD)
Liver	-	+++	+
Intestine	-	++	++
Spleen	-	+++	+
Kidney	-	+++	++
Heart	-	-	-
Lung	-	++	+
Brain	-	+	-
Lymphnode (Abdominal)	-	+	-

Normal (-), mild (+), moderate (++) and severe (+++)

significantly ($p<0.05$) higher compared to other groups at 12 h (day 0) post-infection. There were no significant differences ($p<0.05$) in investigated clinical parameters of mice in all groups at day 6 and 7 (Table 1-4). Other clinical signs observed among challenged group ranged between mild and moderate that included lethargy, hunched posture and shallow and rapid respiration.

Gross findings: As documented for clinical signs, the gross lesions were also constantly observed in different visceral organs which included liver, spleen, kidneys, lungs, heart, intestine, abdominal lymph node and brain of experimentally challenged mice with either *C. pseudotuberculosis* or exotoxin (PLD). The degree of their involvement was compared between challenged and control group (Table 5).

Mice in control group did not show any gross and microscopic pathological changes in all different visceral organs throughout the experimental trial.

In contrast, mice challenged with *C. pseudotuberculosis* group showed significant gross lesions in almost all examined visceral organs. Livers, spleens and kidneys were the most consistently involved

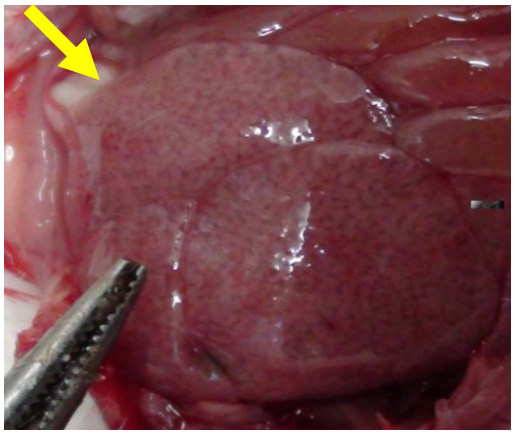

Fig. 5: Liver with mulbery appearance and necrotic lessions (arrow) in intraperitoneally challenged mice with *C. pseudotuberculosis* at day 4 post challenged

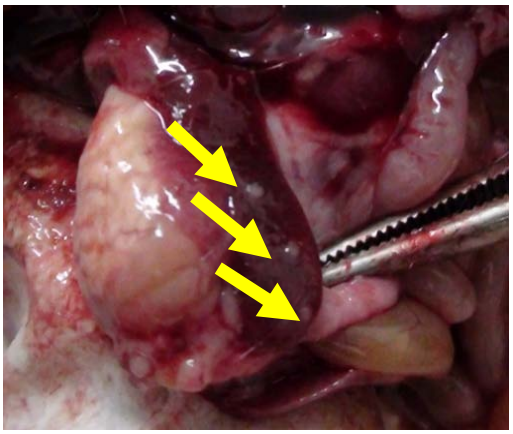

Fig. 6: Spleen with necrotic foci scattered throughout the surface (arrow) in mice infected with *C. pseudtuberculosis* at day 5 post-infcetion

(Table 5). The liver was invariably enlarged, friable and soft in consistency, congested and showed bronze discoloration with necrotic foci scattered throughout surface of the liver (Fig. 5). Similarly, spleen was frequently enlarged and showed constantly necrotic foci scattered through surface of the organs (Fig. 6). Kidneys showed macroabscess, anaemic changes and considerably friability with variable degrees of parenchymatous degeneration and necrotic lesion scattered throughout surface (Fig. 7).

Most of intestine showed edematous swelling, degeneration, cheesy and yellowish color contents particularly in ileum, jejunum and posterior parties of duodenum which were also tinged with slight bloody contents (Fig. 8).

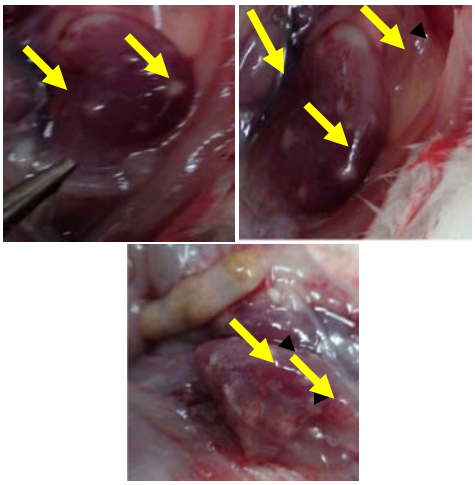

Fig. 7: Kidneys with necrotic lessions in intraperitoneally challenged mice with *C. pseudotuberculosis* at day 5 post infection

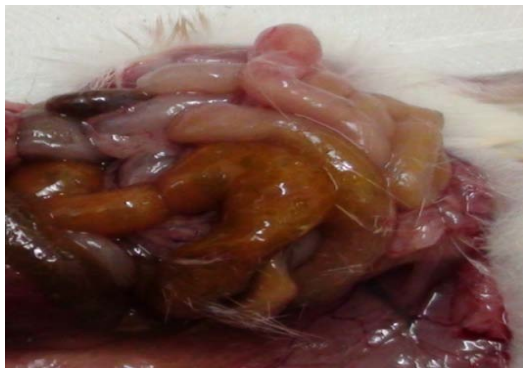

Fig. 8: Intestine with edematous swelling, cheesy and yellowish color contents tinged with slight bloody contents in mice challenged *C. pseutuberculosis* at day 3 post-infection (pi)

Mice in exotoxin group showed gross changes characterized by severe hemorrhages in vast majority of their investigated organs. Intestine organs of this group showed predominantly hemorrhagical changes, odematous swelling degeneration and bloody contents in intestines (Fig. 9). Other gross lesion observed among this group included anaemic changes in kidney.

Mortality rates: No death was recorded throughout the experimental trial in control group. In contrast, animals within whole bacterium group started to die frequently after 24 h where mortality rate reached its peak at 48 h (17.00%) before it started to decline at 50 h and remained in decreased until 120 h post-infection. Deaths were associated with systemic bacteraemia caused by the challenge strain.

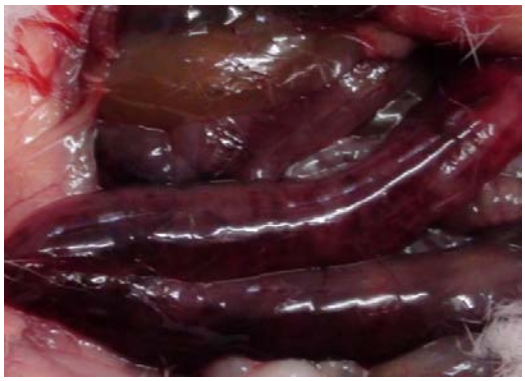

Fig. 9: Intestine with edematous swelling degeneration, bloody color contents tinged with bloody contents in exotoxin group at day 2 post-infection (pi)

Table 6: Mortality rates of mice experimentally challenged with *C. pseudotuberculosis* and its exotoxin (PLD)

	Groups		
Time (h)	Control (%)	*C. pseudotuberculosis* (%)	Toxin (PLD) (%)
12	0	0.00	0.00
24	0	0.00	2.38
48	0	16.70	22.22
72	0	8.33	10.00
96	0	5.00	6.25
120	0	0.00	0.00
144	0	0.00	0.00
168	0	0.00	0.00
Total (%)	0	18.75	25.00

In the mice given extracted exotoxin (PLD) from *C. pseudotuberculosis*, deaths were observed after 12 h where mortality rate reached its peak within 48 h (22.00%) (Table 6). The mortality rate suddenly decreased after 72 h. The total mortality rate of exotoxin group was significantly ($p<0.05$) higher than that observed of *C. pseudotuberculosis* (Table 6). Deaths were associated with systematic toxaemia caused by the exotoxin extracted from the organism.

Histopathology: Briefly, almost all collected organs of infected groups (*C. pseudotuberculosis* and exotoxin groups) were microscopically inflamed and necrotized with the presence of many cytes. Tissues of these infected groups (whole bacterium and exotoxin groups) were congested, oedematous and mixed with inflammatory cells. Mice in control group were healthy and no lesions were found in their organs.

The challenged groups, however, showed histopathological changes of varying degrees. The most pronounced of which were observed on organs that included liver, kidney, lungs, spleen, heart and abdominal lymph nodes. Microscopically, formation of abscess with

signs of calcification and infiltration of macrophages were the most prominently observed in multiple visceral organs of the infected mice.

The lungs, heart, kidney, liver and spleen of mice infected with *C. pseudotuberculosis* showed caseous abscess. There were the presence of tuberculous granuloma (caseating tubercule), giant multinucleated cells, infiltration of neutrophils and macrophages, degeneration, vacuolation (necrosis), haemorrhage and formation of microabscesses (Fig. 10-14).

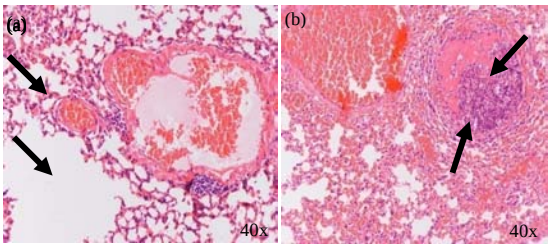

Fig. 10: Lung sections of mice infected intraperitoneally with *C. pseudotuberculosis*: a) Presence of diffuse haemorrhage and severe emphysema (arrows) at day 4 post-infection (hematoxylin and eosin (H&E), x40); b) Presence of capsulated abscess and necrosis (arrows) (H&E, x40)

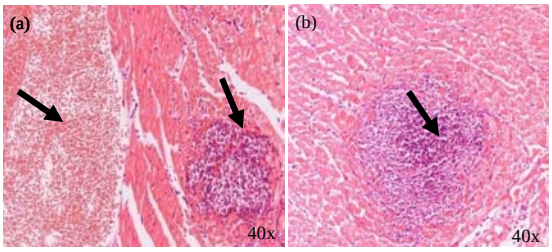

Fig. 11: Heart of mice infected intraperitoneally with *C. pseudotuberculosis*: a) Heart sections showing abscess formation and extensive haemorrhage at day 3 pi. b) Heart sections presenting similar abscess formation at day 4 pi (HE, x40)

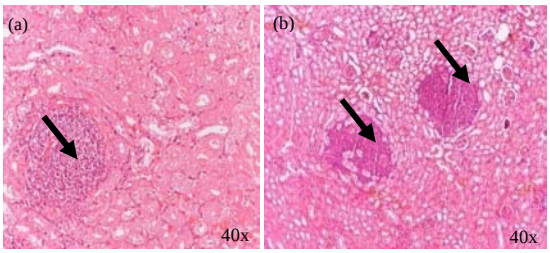

Fig. 12: Photomicrograph of a kidney section showing focal abscesses and severe tubular degeneration a) at day 4 and b) day 6 following infection with *C. pseudotuberculosis*

In contrast, the internal organs of exotoxin group were mostly characterized by severe haemorrhage, generalized congestion and moderate cellular infiltration (Fig. 15 and 16).

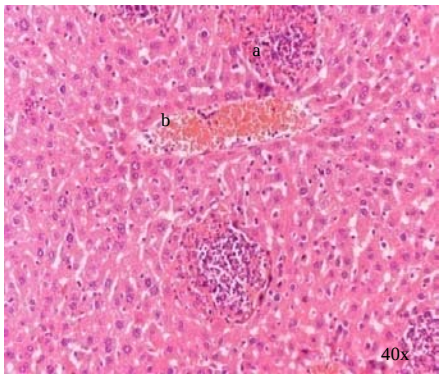

Fig. 13: Photomicrograph of a liver section showing focal areas of abscess formation a and severe congestion b of the central vein at day 4, following infection with *C. pseudotuberculosis*

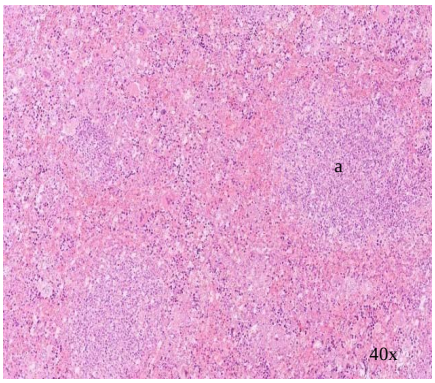

Fig. 14: Photomicrograph of a splenic section showing formation of abscess at day 7 a following infection with *C. pseudotuberculosis*

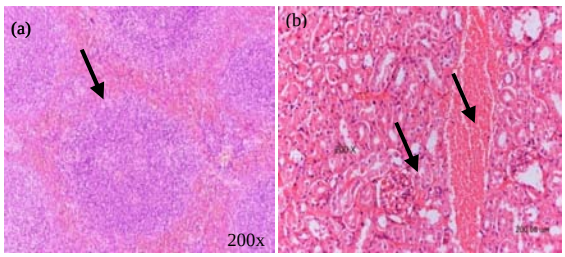

Fig. 15: Visceral organs of mice infected intraperitoneally with exotoxin (PLD) of *C. pseudotuberculosis*: a) showing spleenic proliferation with increased sizes of germinal center (Arrow). b) Liver showing diffuse hemorrhage, severe congension (arrows) and early stage of cellular degeneration

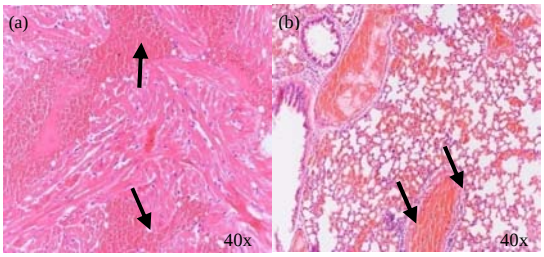

Fig. 16: Photomicrograph of visceral organs of mice infected intraperitoneally with exotoxin (PLD) of *C. pseudotuberculosis*: a) Heart with extensive hemorrhage; b) Lung: Severe congestion and diffuse hemorrhage with increase of inflammatory cells (HE, x40)

The present study reports for the first time, the clinico-pathological changes of mice experimentally infected intraperitoneally with whole bacterium and exotoxin (PLD) extracted from *C. pseuodotuberculosis*. The severity was compared with control group challenged with PBS. Inoculation of exotoxin and whole bacterium of this organism induced clinical symptoms that were relatively tropical characteristic of CLA which included depression, anorexia, submandibular oedema, diarrhea, ruffled coat, eye discharges, poor general condition and occasionally partial tremor of hindquarters. The severity of these symptoms varied with time and character of inoculums. In the study, mice infected intraperitoneally with exotoxin (PLD) developed clinical disease and started to die in the early phase of infection compared to those challenged with whole bacterium group. On the other hand, mice in control group neither suffered from clinical disease nor did they have identifiable gross and histopathological changes in their organs, even though lungs revealed histopathologically mild hemorrhages. This could be explained to the type of euthanasia conducted in the study. However, clinical symptoms from inoculation of exotoxin were more severe in the first 2 days than those caused by inoculation with whole bacterium of *C. pseudotuberculosis* possibly due to difference of character innoculum. In the study, researchers have also noticed that exotoxin (PLD) produced by *C. pseudotuberculosis* was frequently responsible for high mortality rates where most of these mortalities associated with septicaemia caused by this exotoxin. Factors that promote development of endogenous septicaemia following the inoculation may include bacterial colonization and overgrowth in the body, compromised immune defenses and disruption of the mucosal epithelium. In agreement with Nairn *et al*. (1977), this virulence factor (PLD) attributed to the spread of the bacteria from the initial site of infection to secondary sites within the host. The importance of this PLD to the dissemination of *C. pseudotuberculosis* within the host has been illustrated in protection studies (Nairn *et al*., 1977).

In gross findings, researchers have shown a major difference between mice treated with exotoxin and those challenged with whole bacterium. Based on the gross evidence obtained in the study it seems that the inoculums character of the organism was responsible for these differences. In animals treated with exotoxin (PLD), generalized hemorrhage and oedema were noted grossly in all visceral organs of these animals. Small capillaries and blood vessels were engorged with blood and RBCs were also seen in the intravascular areas. Among other internal organs, intestines were the most exclusively congested where cheesy core particularly in the small intestine which in most cases was also tinged with blood. Microscopic observations also indicated a greater severity of damage to the mucosa of all tissues examined. These data support the theory that the exotoxin is believed to promote the bacterial dissemination by increasing vascular permeability following infection. In contrast, mice infected intraperitoneally with whole bacterium allowed an initial bacterial proliferation resulting in extensive abscess formation, particularly in the spleen, liver and kidneys. The number and granuloma area of these lesions increased progressively with time. In gross lesion scores of various visceral organs, the control group showed no identifiable gross lesions. However, the challenged groups, moderate to severe lesions were recorded.

Gross scores of (+) were associated with mild odema, degeneration and capillary proliferation. Lesions of (++) and (+++) were characterized by foci necrosis, increased exuadate and severe congestion. The lesions were focal and multifocal and did appear single or multiple surface of the specific organ. Macroabcess and necrotic lessions scattered through the surface of visceral organs were the features of *C. pseudotuberculosis* induced mice. The absence of well-defined nodules or foci necrosis from internal organs of mice infected with exotoxin (PLD) in this experiment illustrated that the risk of abscess formation relied on the presence of viable bacteria in the animal. Therefore, gross evaluation alone could be useful in studies of mice modeled *C. pseudotuberculosis*. Even though, it seems impractical but histological evaluation may also contribute to the gross observations and provide better insight into the pathogenesis of the lesions. Thus, in the study, histopathological lesion scoring in different visceral organs was also studied. The microscopic findings from the visceral organs of the

infected mice were similar to those described by Jesse *et al.* (2008). But the severity of lesions observed in this study was higher in the infected mice with whole bacterium as compared with exotoxin infected ones in this experiment. Nevertheless, this study described for the first time the detailed histopathology of various visceral organs of mice either following whole bacterium and exotixn (PLD) exposure of *C. pseudotuberculosis*.

However, tissues of these infected animals (mice and whole bacterium groups) had a range of histological lesions that correlated with gross lesion scores. Only the lesion scoring of control group remained unchanged throughout the experiment. The challenged groups presented moderate to severe lesions to their visceral organs.

Moreover, there was histological evidence of abscess formation observed microscopically in almost all examined organs. Abscess formation following infection in these tissues often was regarded as evidence of *C. pseudotuberculosis* infection. The specifity of such structures of *C. pseutuberculosis* infection was also reported in other experiment (Jesse *et al*., 2008). The liver, heart, kidney and lung, of mice inoculated with *C. pseudotuberculosis* showed predominantly, presence of tuberculous granuloma (caseating tubercule), infiltration of neutrophils and macrophages, degeneration, vacuolation (necrosis), haemorrhage and formation of microabscesses. Similarly, the microscopic pathology was similar to those challenged with exotoxin (PLD) but the severity of lesions observed in their organs was milder as compared with mice infected with *C. pseudtuberculosis*.

CONCLUSION

A critical mass of knowledge in clinico-pathological changes of CLA has been required over recent years so that there is now sufficient understanding of the pathological alteration and clinical manifestations induced by *C. pseudotuberculosis* and its exoxtoxin (PLD). Whole bacterium and exotoxin (PLD) intervention are considered important components for vaccine production and prevention strategy for casous lymphadenitis. This study has therefore, documented several abnormalities in almost all investigated parameters in whole bacterium and exotoxin (PLD) challenged mice and has pointed to the significance of this information in both establishing base line data and to control these abnormalities for future clinical settings.

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