Journal of Animal and Veterinary Advances 11 (24): 4660-4667, 2012
ISSN: 1680-5593

Haematological and Biochemical Alterations in Mice Following Experimental Infection with Whole Cell and Exotoxin (PLD) Extracted from *C. Pseudotuberculosis*

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Abstract: *Corynebacterium pseudotuberculosis* is the etiological agent of caseous lymphadenitis, a chronic infectious disease of sheep and goats. Despite the growing interest of this organism, little is known about the causality and effect of this organism on haematological and biochemical values. The purpose of this research, therefore was to determine haematological and serum biochemical features in mice inoculated with whole bacterium and exotoxin (PLD) extracted from *C. pseudotuberculosis*. The study was performed on apparently healthy mice of 2-3 weeks old (n = 64). The mice were divided equally into 3 groups; namely whole bacterium, exotoxin (PLD) and control group. Mice of whole bacterium group were exposed intraperitoneally to 1 mL of the inoculums containing 10^9 Colony-Forming Unit (CFU)/mL of live *C. pseudotuberculosis*. Exotoxin group were exposed intraperitoneally with a single dose of exotoxin (PLD) extracted from *C. pseudotuberculosis*. Mice served as a control group were challenged intraperitoneally to 1 mL of Phosphate-Buffered Saline (PBS), pH 7. Following post-infection, the mice were subjected for blood sample collection using cardiac puncture method for haematological and biochemical analysis. The results of this study revealed that there were significant decrease ($p<0.05$) in Hb, thrombocytes count and significant increase in WBC, neutrophils, monocytes counts in the infected group. Biochemically, there were highly significant increase ($p<0.05$) in the mean levels of CK, AST, ALP and ALT. The mean level of albumin in exotoxin group was significantly lower ($p<0.05$) compared to *C. pseudotuberculosis* and control groups while globulin level was significantly higher in *C. pseuodotuberculosis* group after 48 h. In serum electrolytes, mean level of potassium and phosphate were significantly ($p<0.05$) higher in infected groups compared to control group while there was no significant ($p<0.05$) difference in the levels of sodium, calcium and chloride. In conclusions, the present study of experimental nature showed that *C. pseudotuberculosis* and its exotoxin (PLD) cause disturbances in blood factors and electrolytes and pointed out that t hese haematological and biochemical alterations should be taken into account in the context of diagnosis and treatment of valuable infected hosts in order to prevent further consequences.

Key words: *C. pseudotuberculosis*, exotoxin (PLD), intraperitoneal exposure, mice, thrombocytes

INTRODUCTION

Corynebacterium pseudotuberculosis is the causative agent of chronic infections in wide range of mammalian hosts. Although, many of these infections are of veterinary importance but the most important significant of which is Caseous Lymphadenitis (CLA), a disease of considerable economic importance which primarily affects small ruminants (Dorella *et al*., 2006; Baird and Fontaine, 2007). The significance of this disease is evidenced by the global documentation and political debate which has been ongoing for over a century. Beside its economic importance on both animal productions and productivity, the disease is also potentially zoonotic where many cases of human infection have been reported on several occasions (Lopez *et al*., 1996; Hamilton *et al*., 1968; Hill *et al*., 1978; Mills *et al*., 1997; Peel *et al*., 1997; Jain-Lambert *et al*., 2006). However, in Malaysia, the disease is known to be common in sheep-goat rearing farms with semi-extensive management practices and was first described in the early sixties (http://agrolink.moa.my/jph). In this context, mouse model has been used to study the haematological and biochemical alterations caused by whole cell and exotoxin

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(PLD) extracted from *C. pseudotuberculosis*. There was no prior knowledge of haematological and biochemical parameters in these models. Thus, the aim of this study was evaluating the potential of these parameters as well as establishing a base line data that might be useful for the actual host of this zoonotic disease (CLA).

MATERIALS AND METHODS

Animals, experimental design, samples collection: Sixty four mice, 2-3 weeks old were used in this study. The mice were kept under standard condition for 2 weeks prior the experiment for acclimatization. The infection was carried out in three groups; namely whole bacterium, exotoxin and control groups. The whole bacterium and exotoxin (PLD) groups were intraperitoneally challenged through a single inoculation of 10^9 cfu *C. pseudotuberculosis* and exotoxin (PLD), respectively. The third group was similarly inoculated with PBS and served as a control group. Three animals from each group were sacrificed and blood samples were collected by cardiac puncture using a 26 G x 1.5 Venoject needle (PrecisionGlide™, Becton Dickinson, UK) with venoject holder (Vacutainer®, BD vacutainer™, USA) for haematological and biochemical tests on the day of inoculation and then on 2, 3, 4, 5, 6 and 7 days Post-Infection (PI). All procedures and experiments described were undertaken under a project license approved by Animal Utilization Protocol Committee with reference number: UPM/FPV/PS/3.2.1.551/AUP-R120.

Bacterial strain and culture condition: *C. pseudotuberculosis* was earlier isolated from animals with gross visible lesions of CLA at TPU farm, Universiti Putra Malaysia (Jesse *et al*., 2008). The organism was then sub-cultured onto 10% sheep blood agar and MacConkey's agar after which the plates were incubated at 37°C for 48 h aerobically as well as in 5% CO_2 incubator. Cultures with bacterial growth were microscopically examined and biochemically identified.

Exotoxin (PLD): PLD was extracted as described by Zaki (1968).

Statistical analysis: Statistical analysis was performed using PASW Statistics 18, Release Version 18.0.0 (= D3 SPSS, Inc., 2009, Chicago, IL. After testing normal distributions of the data, one way Analysis of Variance (ANOVA) was used with Duncan post hoc multiple comparisons to investigate significant differences between control and infected groups at $p<0.05$.

RESULTS AND DISCUSSION

Haematological and biochemical findings: The mean values of blood indices are presented in Table 1-16. Within the challenged and non challenged groups, the mean levels of MCV and MCHC were significantly higher ($p<0.05$) at day 3, 4, 5 and 6 in *C. pseudotuberculosis* group compared to control and exotoxin group (Table 1). The haemoglobin level was only significant at day 5, 6 and 7 in the *C. pseudotuberculosis* group ($p<0.05$) compared to the control and exotoxin group (Table 2). While the mean levels of RBC and WBCs were significantly higher at day 1, 2 and 3 in exotoxin group compared to control and *C. pseudotuberculosis* group (Table 3). However, the mean level of platelets differed significantly ($p<0.05$) at day 2, 3 and 4 both in *C. pseudotuberculosis* and exotoxin group compared to control group (Table 4). The mean level of monocytes and lymphocytes were only significant in the exotoxin group (Table 5) while the mean level of neutrophils differed significantly both in *C. pseudotuberculosis* and exotoxin group compared to control group (Table 4). There were no significant differences ($p<0.05$) in the levels of baso and eosinophils between the challenged and control groups (Table 6). Exotoxin group had significantly higher mean levels of CK and AST compared to *C. pseudotuberculosis* and control group (Table 7). However, mean of ALT in exotoxin group differed significantly ($p<0.05$) through experimental period while mean of ALT in

Table 1: MCV and MCHC of experimentally challenged and non challenged mice (Mean±SD)

	Parameters					
	MCV (f/L)			MCHC (g/L)		
Time (h)	Control	*C. pseudotuberculosis*	Exotoxin (PLD)	Control	*C. pseudotuberculosis*	Exotoxin (PLD)
12	35.22±2.17^{a,x}	38.15±3.33^{a,x}	38.19±3.04^{a,x}	438.10±34.21^{a,x}	413.55±46.04^{a,x}	419.58±34.37^{a,x}
24	35.20±2.92^{a,x}	37.97±2.57^{a,x}	40.59±2.92^{b,y}	438.10±34.21^{a,x}	399.57±14.60^{b,x}	424.94±41.72^{a,x}
48	34.80±3.43^{a,x}	48.20±0.95^{b,y}	38.43±4.26^{a,x}	422.76±22.28^{a,x}	322.92±10.95^{b,y}	406.54±46.82^{a,x}
72	35.20±2.92^{a,x}	49.87±4.44^{b,y}	35.53±1.16^{a,x}	438.10±34.21^{a,x}	318.67±11.30^{b,y}	411.75±24.86^{a,x}
96	35.20±2.92^{a,x}	45.78±7.21^{b,y}	33.99±0.98^{a,x}	438.10±34.21^{a,x}	440.04±11.68^{a,x}	431.32±13.43^{a,x}
120	35.00±2.85^{a,x}	40.62±2.01^{b,y}	33.84±1.78^{a,x}	438.10±34.21^{a,x}	390.74±17.72^{b,y}	457.33±12.86^{a,x}
144	35.20±2.92^{a,x}	40.52±3.11^{b,y}	39.49±2.04^{a,x}	439.41±32.63^{a,x}	398.11±13.91^{b,y}	424.34±20.43^{a,x}
168	35.20±2.92^{a,x}	38.98±1.27^{a,x}	35.20±2.92^{a,x}	438.10±34.21^{a,x}	395.91±18.77^{b,y}	404.50±16.25^{a,x}

^{a,b}Means with different superscripts with in row differed significantly ($p<0.05$) due to treatment effect; ^{x,y}Means with different superscripts with in column differed significantly ($p<0.05$) due to time effect

Table 2: Hb and PCV of experimentally challenged and non challenged mice during the experimental period (Mean±SD)

	Parameters					
	Hb (g/L)			PCV(l/L)		
Time (h)	Control	*C. pseudotuberculosis*	Exotoxin (PLD)	Control	*C. pseudotuberculosis*	Exotoxin (PLD)
12	130.60±17.22^{a,x}	131.80±11.120^{a,x}	182.40±9.070^{b,y}	0.30±0.04^{a,x}	0.32±0.02^{a,x}	0.43±0.06^{b,y}
24	130.20±16.48^{a,x}	120.20±6.9000^{a,x}	177.20±12.55^{b,z}	0.31±0.03^{a,x}	0.30±0.01^{a,x}	0.46±0.03^{b,z}
48	133.20±13.31^{a,x}	120.00±9.3000^{a,x}	154.60±19.88^{a,x}	0.31±0.05^{a,x}	0.37±0.03^{a,x}	0.38±0.04^{a,x}
72	130.56±17.23^{a,x}	129.00±9.4000^{a,x}	154.00±20.50^{a,x}	0.30±0.04^{a,x}	0.40±0.04^{b,x}	0.37±0.04^{a,x}
96	132.80±17.85^{a,x}	130.80±23.103^{a,x}	138.00±16.50^{a,x}	0.31±0.05^{a,x}	0.29±0.05^{a,x}	0.32±0.03^{a,x}
120	136.60±13.44^{a,x}	109.80±5.8400^{b,y}	136.40±13.66^{a,x}	0.30±0.04^{a,x}	0.28±0.02^{a,x}	0.31±0.03^{a,x}
144	131.20±18.59^{a,x}	102.18±19.530^{b,y}	109.68±16.35^{a,x}	0.30±0.04^{a,x}	0.25±0.06^{a,x}	0.25±0.03^{a,x}
168	130.60±16.00^{a,x}	87.50±23.020^{b,z}	106.68±12.65^{a,x}	0.31±0.06^{a,x}	0.23±0.04^{a,x}	0.25±0.03^{a,x}

Table 3: RBCs and WBCs of experimentally challenged and nonchallenged mice (Mean±SD)

	Parameters					
	RBCs ($\times10^{12}$/L)			WBCs ($\times10^{9}$/L)		
Time (h)	Control	*C. pseudotuberculosis*	Exotoxin (PLD)	Control	*C. pseudotuberculosis*	Exotoxin (PLD)
12	8.51±1.14^{a,x}	8.40±0.42^{a,x}	11.32±0.77^{b,y}	2.68±1.48^{a,x}	5.88±2.630^{a,x}	23.67±12.42^{b,z}
24	7.72±0.97^{a,x}	7.90±0.41^{a,x}	11.35±0.76^{b,y}	3.16±1.48^{a,x}	4.33±2.110^{a,x}	14.72±5.660^{b,y}
48	8.51±1.14^{a,x}	7.92±0.69^{a,x}	9.99±1.26^{a,x}	2.55±1.42^{a,x}	5.75±1.850^{a,x}	16.92±18.03^{b,y}
72	8.52±1.13^{a,x}	8.17±1.03^{a,x}	10.53±1.34^{b,y}	2.69±1.46^{a,x}	3.79±1.800^{a,x}	9.73±4.680^{b,y}
96	8.51±1.14^{a,x}	8.84±1.29^{a,x}	9.41±1.10^{a,x}	2.68±1.48^{a,x}	3.75±1.470^{a,x}	7.45±2.300^{b,y}
120	8.06±1.22^{a,x}	6.93±0.53^{a,x}	9.07±0.94^{a,x}	2.12±1.70^{a,x}	6.09±2.750^{b,y}	4.26±2.500^{a,x}
144	7.72±0.97^{a,x}	6.39±1.49^{a,x}	6.92±1.41^{a,x}	2.69±1.47^{a,x}	13.34±17.39^{b,z}	2.45±1.390^{a,x}
168	8.15±0.82^{a,x}	6.08±1.39^{b,x}	6.92±1.41^{a,x}	2.62±1.30^{a,x}	13.86±2.780^{b,z}	2.45±1.390^{a,x}

Table 4: Thrombocytes and neutrophils of experimentally challenged and non challenged mice (Mean±SD)

	Parameters					
	Thromb ($\times10^{9}$/L)			Neu ($\times10^{9}$/L)		
Time (h)	Control	*C. pseudotuberculosis*	Exotoxin (PLD)	Control	*C. pseudotuberculosis*	Exotoxin (PLD)
12	675.06±435.29^{a,x}	895.60±230.47^{a,x}	328.80±142.97^{a,x}	0.58±0.43^{a,x}	3.08±1.90^{b,x}	11.28±8.12^{c,z}
24	656.46±456.06^{a,x}	820.88±439.06^{a,x}	190.60±83.390^{b,y}	0.79±0.59^{a,x}	2.32±0.96^{b,x}	4.81±1.21^{b,y}
48	668.79±433.59^{a,x}	58.74±36.990^{b,y}	179.52±197.11^{c,y}	0.58±0.43^{a,x}	3.92±1.01^{b,x}	6.02±6.28^{a,x}
72	671.26±437.26^{a,x}	33.32±16.340^{b,z}	95.78±23.840^{c,x}	0.79±0.59^{a,x}	1.62±1.32^{a,y}	4.98±2.96^{b,y}
96	650.80±425.69^{a,x}	65.54±20.690^{b,y}	136.00±80.290^{c,x}	0.58±0.43^{a,x}	2.83±1.05^{b,x}	3.94±1.48^{b,y}
120	676.86±431.46^{a,x}	62.12±49.250^{b,y}	177.38±87.110^{c,x}	0.65±0.44^{a,x}	3.66±1.74^{b,x}	2.61±1.70^{b,y}
144	680.26±429.62^{a,x}	259.70±480.00^{a,x}	93.88±79.530^{a,x}	0.61±0.49^{a,x}	6.19±1.00^{b,y}	0.42±0.19^{a,x}
168	672.90±437.74^{a,x}	719.76±886.03^{a,x}	93.88±79.530^{a,x}	0.58±0.43^{a,x}	8.84±2.29^{b,z}	0.43±0.27^{a,x}

Table 5: Monocytes and lymphocytes of experimentally challenged and non challenged mice (Mean±SD)

	Parameters					
	Mono ($\times10^{9}$/L)			Lymph ($\times10^{9}$/L)		
Time (h)	Control	*C. pseudotuberculosis*	Exotoxin (PLD)	Control	*C. pseudotuberculosis*	Exotoxin (PLD)
12	0.08±0.04^{a,x}	0.29±0.13^{a,x}	1.08±0.56^{b,z}	1.84±0.93^{a,x}	2.84±1.09^{a,x}	9.32±4.01^{b,z}
24	0.14±0.07^{a,x}	0.29±0.22^{a,x}	0.55±0.11^{b,y}	2.07±0.90^{a,x}	2.03±0.89^{a,x}	8.50±4.90^{b,z}
48	0.08±0.04^{a,x}	0.38±0.19^{a,x}	1.35±1.73^{b,z}	1.83±0.94^{a,x}	1.97±0.36^{a,x}	8.07±8.13^{b,z}
72	0.14±0.07^{a,x}	0.29±0.12^{a,x}	0.85±0.58^{b,y}	2.03±0.88^{a,x}	1.66±0.66^{a,x}	3.30±1.23^{a,y}
96	0.08±0.04^{a,x}	0.31±0.18^{a,x}	0.37±0.21^{b,y}	1.86±0.93^{a,x}	0.78±0.30^{a,x}	2.80±1.13^{a,x}
120	0.14±0.07^{a,x}	0.34±0.16^{b,x}	0.20±0.10^{a,x}	1.89±0.71^{a,x}	1.81±1.00^{a,x}	1.25±0.49^{a,x}
144	0.11±0.02^{a,x}	0.86±0.23^{b,y}	0.10±0.06^{a,x}	1.64±0.82^{a,x}	2.68±1.21^{a,x}	1.84±1.11^{a,x}
168	0.10±0.05^{a,x}	1.07±0.41^{b,z}	0.06±0.04^{a,x}	1.67±0.71^{a,x}	3.22±0.48^{b,y}	1.41±0.58^{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 time effect

C. pseudotuberculosis differed significantly ($p<0.05$) at 4, 5, 6 and 7 compared to control group (Table 8). Mean levels ALP in exotoxin group differed significantly ($p<0.05$) at day 1, 2 and 3 compared to *C. pseudotuberculosis* and control group (Table 8) while there was no significant difference in the mean level of GGT between challenged and non-challenged groups (Table 9). However, there were variations in the level of

Table 6: Esinophils and basophils of experimentally challenged and non challenged mice (Mean±SD)

	Parameters					
	Eosino ($\times10^9$/L)			Baso ($\times10^9$/L)		
Time (h)	Control	*C. pseudotuberculosis*	Exotoxin (PLD)	Control	*C. pseudotuberculosis*	Exotoxin (PLD)
12	0.10±0.10$^{a, x}$	0.13±0.11$^{a, x}$	0.43±0.29$^{a, x}$	0.00±0.00$^{a, x}$	0.00±0.00$^{a, x}$	0.00±0.00$^{a, x}$
24	0.20±0.11$^{a, x}$	0.22±0.19$^{a, x}$	0.16±0.05$^{a, x}$	0.00±0.00$^{a, x}$	0.00±0.00$^{a, x}$	0.00±0.00$^{a, x}$
48	0.15±0.07$^{a, x}$	0.12±0.04$^{a, x}$	0.55±0.78$^{a, x}$	0.00±0.00$^{a, x}$	0.03±0.05$^{a, x}$	0.03±0.05$^{a, x}$
72	0.20±0.11$^{a, x}$	0.14±0.14$^{a, x}$	0.13±0.05$^{a, x}$	0.00±0.00$^{a, x}$	0.01±0.02$^{a, x}$	0.00±0.01$^{a, x}$
96	0.10±0.10$^{a, x}$	0.03±0.03$^{a, x}$	0.09±0.03$^{a, x}$	0.00±0.00$^{a, x}$	0.00±0.00$^{a, x}$	0.00±0.00$^{a, x}$
120	0.20±0.11$^{a, x}$	0.09±0.08$^{a, x}$	0.08±0.10$^{a, x}$	0.00±0.00$^{a, x}$	0.00±0.00$^{a, x}$	0.00±0.00$^{a, x}$
144	0.11±0.07$^{a, x}$	0.13±0.07$^{a, x}$	0.06±0.05$^{a, x}$	0.00±0.00$^{a, x}$	0.00±0.00$^{a, x}$	0.00±0.00$^{a, x}$
168	0.11±0.07$^{a, x}$	0.13±0.02$^{a, x}$	0.10±0.07$^{a, x}$	0.00±0.00$^{a, x}$	0.00±0.00$^{a, x}$	0.00±0.00$^{a, x}$

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

Table 7: Creatine Kinase (CK) and Aspartate Aminotransferase (AST) of e challenged and non challenged mice (Mean±SD)

	Parameters					
	CK (U/L)			AST (U/L)		
Time (h)	Control	*C. pseudotuberculosis*	Exotoxin (PLD)	Control	*C. pseudotuberculosis*	Exotoxin (PLD)
12	414.00±125.42	265.8±164.250$^{a, x}$	10294.6±6036.990$^{b, z}$	156.12±11.03$^{a, x}$	136.64±59.350$^{a, x}$	1995.86±217.870$^{b, x}$
24	412.00±120.44$^{a, x}$	79.2±20.3200$^{a, x}$	4330.2±2701.230$^{b, y}$	162.78±7.310$^{a, x}$	187.58±121.81$^{a, x}$	2060.94±681.650$^{b, x}$
48	413.60±124.89$^{a, x}$	220.8±129.440$^{a, x}$	3476.8±2725.360$^{b, y}$	162.58±13.92$^{a, x}$	751.98±225.83$^{b, x}$	2522.98±1240.55$^{c, x}$
72	440.20±146.70$^{a, x}$	557.2±245.730$^{a, x}$	17477.4±28172.74$^{b, x}$	156.32±13.41$^{a, x}$	961.84±438.35$^{b, x}$	2156.30±1241.90$^{c, x}$
96	412.18±125.21$^{a, x}$	3559.2±2502.40$^{b, x}$	1456.8±1516.250$^{c, x}$	153.44±10.40$^{a, x}$	1526.92±398.95$^{b, x}$	757.48±433.580$^{c, x}$
120	414.00±125.42$^{a, x}$	4505.8±5433.70$^{b, x}$	356.6±263.5100$^{c, x}$	150.90±10.78$^{a, x}$	1296.98±940.78$^{b, x}$	243.46±270.800$^{a, x}$
144	414.00±125.42$^{a, x}$	2052.6±1674.79$^{b, x}$	4466.6±7456.180$^{c, x}$	156.24±11.52$^{a, x}$	2383.24±296.01$^{b, x}$	1262.74±650.600$^{c, x}$
168	414.00±124.75$^{a, x}$	658.8±651.670$^{a, x}$	4466.6±7456.180$^{c, x}$	151.82±9.210$^{a, x}$	377.12±267.30$^{b, x}$	1084.20±650.570$^{c, x}$

Table 8: Alanine Transaminase (ALT) and Alkaline Phosphatase (ALP) of challenged and non challenged mice (Mean±SD)

	Parameters					
	ALT (U/L)			ALP (U/L)		
Time (h)	Control	*C. pseudotuberculosis*	Exotoxin (PLD)	Control	*C. pseudotuberculosis*	Exotoxin (PLD)
12	47.40±18.80$^{a, x}$	44.06±15.820$^{a, x}$	636.70±402.06$^{b, z}$	126.80±14.80$^{a, x}$	76.80±8.780$^{a, x}$	334.60±152.64$^{b, y}$
24	49.94±18.25$^{a, x}$	51.10±16.700$^{a, x}$	883.36±377.24$^{b, z}$	126.00±39.35$^{a, x}$	75.60±19.34$^{a, x}$	257.00±84.730$^{b, y}$
48	47.38±17.70$^{a, x}$	68.12±15.070$^{a, x}$	1200.90±52.900$^{b, z}$	120.40±9.200$^{a, x}$	90.00±13.63$^{a, x}$	242.40±149.47$^{b, y}$
72	47.40±18.28$^{a, x}$	499.16±287.54$^{b, x}$	479.52±330.56$^{b, y}$	127.16±15.01$^{a, x}$	113.60±44.25$^{a, x}$	250.40±93.540$^{b, y}$
96	47.76±18.40$^{a, x}$	763.82±67.440$^{b, x}$	163.40±88.080$^{c, z}$	122.66±15.10$^{a, x}$	171.20±74.77$^{a, x}$	165.00±49.910$^{a, x}$
120	36.76±10.21$^{a, x}$	357.98±271.18$^{b, x}$	83.48±63.100$^{c, z}$	126.54±14.34$^{a, x}$	93.20±33.92$^{a, x}$	72.60±38.900$^{a, x}$
144	47.54±18.77$^{a, x}$	710.88±270.27$^{b, x}$	297.68±291.36$^{b, x}$	124.52±16.77$^{a, x}$	113.60±40.63$^{a, x}$	87.20±24.590$^{a, x}$
168	47.80±17.74$^{a, x}$	172.82±213.99$^{b, x}$	136.64±119.48$^{b, x}$	126.80±14.94$^{a, x}$	90.00±34.40$^{a, x}$	82.40±20.830$^{a, x}$

$^{a-c}$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 time effect

Table 9: Gamma-Glutamyl Transferase (GGT) of challenged and non challenged mice (Mean±SD)

	Parameters		
	GGT (U/L)		
Time (h)	Control	*C. pseudotuberculosis*	Exotoxin (PLD)
12	2.9±0.0$^{a, x}$	2.90±0.00$^{a, x}$	3.38±0.90$^{a, x}$
24	2.9±0.0$^{a, x}$	2.90±0.00$^{a, x}$	7.98±3.35$^{a, x}$
48	2.9±0.0$^{a, x}$	2.90±0.00$^{a, x}$	18.60±5.36$^{a, x}$
72	2.9±0.0$^{a, x}$	2.90±0.00$^{a, x}$	17.40±8.04$^{a, x}$
96	2.9±0.0$^{a, x}$	3.52±1.38$^{a, x}$	3.52±1.38$^{a, x}$
120	2.9±0.0$^{a, x}$	3.52±1.38$^{a, x}$	2.90±0.00$^{a, x}$
144	2.9±0.0$^{a, x}$	3.12±0.49$^{a, x}$	3.34±0.60$^{a, x}$
168	2.9±0.0$^{a, x}$	3.32±0.93$^{a, x}$	3.12±0.49$^{a, x}$

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

certain parameters such as urea between exotoxin and *C. pseudotuberculosis* group within the challenged group. The most striking difference was observed at 48 h where exotoxin group had a signicantly higher mean urea level compared to *C. pseudotuberculosis* and control group. Within challenged group, the mean level of Creatine Kinase (CK) in exotoxin group was significantly different through all experimental period compared to *C. pseudotuberculosis* and control group (Table 7). However, there was no significant differences in the levels of direct and total billurubin ($p<0.05$) between challenged and non challenged group (Table 10). In proteins, the mean level of albumin in exotoxin group was significantly lower ($p<0.05$) compared to *C. pseudotuberculosis* and control groups while globulin level was significantly higher in *C. pseuodotuberculosis* group after 48 h (Table 12). There was no significant difference in the level of total protein among challenged groups. In serum

electrolytes, mean level of potassium and phosphate were significantly higher in challenged groups at day 2, 3 and 4 compared to control group (Table 15) while there was no significant difference in the levels of sodium, calcium and chloride (Table 14 and 16).

Haematologically, the results of this study described clearly for the first time, changes over time in a wide range of hematological and biochemical parameters in mice experimentally infected with whole bacterium and exotoxin extracted from *C. pseudotuberculosis*. The findings from these two groups were compared haematologically and biochemically with control group.

Mice in this study particularly the exotoxin group had severe hemolytic anaemia. In these animals, melana, dark red fluid in body cavities were documented. This was in agreement with other experimental studies of *C. pseudotuberculosis*. Thrombocytopenia was detected in mice infected with whole bacterium and exotoxin (PLD). The severity was much greater in toxin group in the first 24 h than in whole bacterium group. It has been speculated that the severity of thrombocytopenia might be influenced by the severity of the anaemia. The pathogenesis of thrompocytopenia in mice infected with whole bacterium and exotoxin of *C. pseudotuberculosis* remains unclear; however, resolution of normal PLT numbers only in mice treated with whole bacterium in this study by day 8 suggested a failure in megakaryocytic response in bone marrow, supporting increased PLT destruction of mice challenged with exotoin as a likely mechanism. Sequestration of PLTs in the spleen and hypersplenism, a marked primary PLT are both considered rare cause of thrompocytopenia in animals (Russel and Grindem, 2000).

In this study, the results indicated that the WBC, neutrophils and monocytes counts in the infected group were significantly higher than in the control. High elevated levels of serum Creatine (CK) were among the most striking findings observed in the infected mice. A more than 1000 fold increase in blood level of creatine was found in exotoxin and whole bacterium animal. The severity was much higher in exotoxin treated than *C. pseudotuberculosis*. A potential explanation for the highly elevated levels of serum creatine induced by exotoxin (PLD) and whole bacterium (*C. pseudotuberculosis*) would be attributed to heart or skeletal muscle damage (Van Deursen *et al*., 1993). Other most striking findings in this study were increases of serum AST, ALP and ALT concentrations. These enzymes rapidly and intensely increased plasma activity following infection. The elevated levels of these parameters resulted due to liver damage or hepatotoxicity, particularly in hepatocytes, induced by the challenged inoculums. Similar results were reported in wide range of diseases associated with liver damage in sheep (Gonzalo-Orden *et al*., 2003; Phiri *et al*., 2007; Saleh, 2008; Humann-Ziehank *et al*., 2001). Although, GGT activity was present in many tissues, notable elevation in activity in the serum is primarily observed in liver disease. This evidence is confirmed by the current study where levels of GGT considerably increased overtime in infected groups.

Electrolyte imbalances occur commonly because of loss of electrolytes, shifts of certain electrolytes or relative changes in concentrations due to loss of water (Radostits *et al*., 2007). The results indicated that the phosphorus concentration in the infected group were higher than the control group ($p<0.05$). Serum phosphorus values principally reflect current dietary intake (Whitaker *et al*., 1998). Hyperphosphatemia may be due to hypocalcaemia and consist of tetany and soft tissue mineralization (Kaneko *et al*., 2008). Experimental studies indicate that that the electrolyte K concentration does not always reflect K deficits (Brobst, 1986). Changes in K concentration occur in broad range of clinical conditions. Nevertheless, the data showed no significant differences of the sera levels of calcium, sodium, chloride between in infected and non infected ones. This could be explained to the decreased GFR resulting from a renal failure which led to an increase in the fractional clearance of some substances in the urine to maintain clinically normal plasma concentrations of these substances for as long as possible. Similarly, there was also no significant difference in the levels of Hb, PCV between infected and control group. Furthermore, it has to be emphasised that there was also a significant difference ($p<0.05$) in certain parameters included MCV, MSHC, RBCs between the infected and non infected groups. Although, plasma urea is a poor marker of kidney disease in sheep as being an indicator of alimentary protein supply (Wallace *et al*., 2006) but the results indicated increased levels of plasma urea in infected mice with whole bacterium and exotoxin extracted from *C. pseudotuberculosis*. Similar results have also been reported in experimental toxicology in sheep (Fernandez *et al*., 2003). In plasma proteins, albumin concentration declined slightly in mice infected with exotoxin. The decrease in this group specifically may have been an effect of stress and rigorous inappettence which supervened after infection. It is also possible that the damage observed in the liver of these animals influenced

Table 10: Direct Bilirubin (D. Bill) and Total Billuribin (T. Bill) of challenged and non challenged mice (Mean±SD)

	Parameters					
	D. Bill (umol/L)			T. Bill (umol/L)		
Time (h)	Control	*C. pseudotuberculosis*	Exotoxin (PLD)	Control	*C. pseudotuberculosis*	Exotoxin (PLD)
12	0.17±0.34^{a,x}	0.09±0.00^{a,x}	0.90±0.0^{a,x}	1.55±1.41^{a,x}	0.19±0.170^{a,x}	1.05±2.150^{a,x}
24	0.33±0.42^{a,x}	0.09±0.00^{a,x}	0.90±0.0^{a,x}	1.38±1.54^{a,x}	0.17±0.130^{a,x}	2.35±1.730^{a,x}
48	0.17±0.34^{a,x}	0.09±0.00^{a,x}	0.90±0.0^{a,x}	1.35±1.53^{a,x}	2.10±1.430^{a,x}	3.362±0.84^{a,x}
72	0.17±0.34^{a,x}	0.09±0.00^{a,x}	0.90±0.0^{a,x}	1.77±1.33^{a,x}	3.90±2.610^{a,x}	2.91±3.000^{a,x}
96	0.01±0.04^{a,x}	0.09±0.00^{a,x}	0.90±0.0^{a,x}	1.41±1.55^{a,x}	10.50±8.410^{a,x}	5.78±7.820^{a,x}
120	0.17±034^{a,x}	0.09±0.00^{a,x}	0.90±0.0^{a,x}	1.34±1.53^{a,x}	0.65±0.840^{a,x}	0.05±0.040^{a,x}
144	0.17±0.34^{a,x}	0.09±0.00^{a,x}	0.90±0.0^{a,x}	1.55±1.38^{a,x}	0.27±0.400^{a,x}	1.11±1.060^{a,x}
168	0.17±0.34^{a,x}	0.09±0.00^{a,x}	0.90±0.0^{a,x}	1.45±1.53^{a,x}	0.17±0.183^{a,x}	0.33±0.220^{a,x}

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

Table 11: Creatine (Crea) and urea of challenged and non challenged mice (Mean±SD)

	Parameters					
	Crea (umol/L)			Urea (mmol/L)		
Time (h)	Control	*C. pseudotuberculosis*	Exotoxin (PLD)	Control	*C. pseudotuberculosis*	Exotoxin (PLD)
12	44.60±8.23^{a,x}	47.80±2.160^{a,x}	85.60±13.16^{b,y}	8.22±2.14^{a,x}	6.34±1.48^{a,x}	24.88±3.71^{b,y}
24	44.66±8.41^{a,x}	54.80±6.830^{a,x}	95.40±24.38^{b,y}	8.52±2.42^{a,x}	10.74±2.17^{a,x}	30.44±4.33^{b,y}
48	44.56±6.94^{a,x}	73.40±9.780^{a,x}	117.00±31.60^{b,y}	8.22±2.13^{a,x}	15.54±1.82^{b,y}	33.46±6.90^{c,y}
72	44.58±8.30^{a,x}	73.60±13.79^{a,x}	150.40±33.05^{b,y}	8.22±2.64^{a,x}	19.26±5.07^{b,y}	49.94±13.94^{c,z}
96	44.72±6.85^{a,x}	73.60±13.79^{a,x}	99.20±48.70^{b,y}	7.90±1.62^{a,x}	38.38±9.24^{b,z}	52.26±18.70^{c,z}
120	44.62±8.22^{a,x}	62.60±11.82^{a,x}	53.60±15.75^{a,x}	8.04±1.39^{a,x}	22.86±8.89^{b,y}	24.88±14.74^{c,y}
144	44.80±8.49^{a,x}	63.20±13.47^{a,x}	47.40±12.85^{a,x}	8.34±2.25^{a,x}	22.52±4.16^{b,x}	10.80±3.81^{a,x}
168	44.62±8.22^{a,x}	44.40±5.270^{a,x}	42.79±13.52^{a,x}	8.48±2.51^{a,x}	7.98±3.20^{a,x}	9.48±2.72^{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 time effect

Table 12: Albumin (Alb) and Globulin (Glob) of challenged and non challenged mice (Mean±SD)

	Parametrs					
	Alb (g/L)			Glob (g/L)		
Time (h)	Control	*C. pseudotuberculosis*	Exotoxin (PLD)	Control	*C. pseudotuberculosis*	Exotoxin (PLD)
12	35.04±4.41^{a,x}	35.02±0.93^{a,x}	19.18±4.86^{b,x}	18.52±4.50^{a,x}	19.12±2.66^{a,x}	25.98±8.05^{a,x}
24	35.22±4.24^{a,x}	35.70±4.71^{a,x}	19.72±7.25^{b,x}	18.66±4.51^{a,x}	21.56±2.90^{a,x}	21.22±9.50^{a,x}
48	34.88±4.25^{a,x}	39.24±7.51^{a,x}	18.08±3.10^{b,x}	18.02±4.70^{a,x}	29.12±6.41^{b,y}	27.92±4.93^{a,x}
72	35.14±4.33^{a,x}	37.56±4.33^{a,x}	23.12±4.57^{b,x}	19.16±4.61^{a,x}	37.12±3.72^{b,y}	20.32±10.89^{a,x}
96	35.60±3.79^{a,x}	38.18±6.59^{a,x}	21.50±5.92^{b,x}	18.04±4.58^{a,x}	41.54±8.91^{b,z}	26.94±10.26^{a,x}
120	35.28±4.33^{a,x}	28.50±5.96^{a,x}	21.44±4.11^{b,x}	17.50±3.25^{a,x}	33.06±8.52^{b,y}	22.62±5.49^{a,x}
144	34.98±4.49^{a,x}	22.76±1.87^{b,y}	26.00±5.43^{b,x}	18.08±4.07^{a,x}	30.18±3.52^{b,y}	19.46±3.82^{a,x}
168	35.82±3.40^{a,x}	21.14±2.92^{b,y}	26.80±589^{b,x}	18.78±4.45^{a,x}	26.28±2.77^{b,y}	17.36±2.66^{a,x}

^{a,b}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 time effect

Table 13: Total Protein (T. Prot) and ratio of Albumin-Globulin (A:G) of challenged and non challenged mice (Mean±SD)

	Parametrs					
	T. Prot (g/L)			A:G (g/L)		
Time (h)	Control	*C. pseudotuberculosis*	Exotoxin (PLD)	Control	*C. pseudotuberculosis*	Exotoxin (PLD)
12	49.76±3.07^{a,x}	55.26±3.300^{a,x}	42.82±7.890^{a,x}	1.56±0.15^{a,x}	1.83±0.26^{a,x}	0.79±0.47^{b,x}
24	49.50±2.31^{a,x}	58.40±4.350^{b,x}	41.04±3.870^{a,x}	1.62±0.27^{a,x}	1.73±0.22^{a,x}	1.37±1.21^{a,x}
48	49.46±5.09^{a,x}	66.14±8.030^{b,x}	46.58±2.480^{a,x}	1.54±0.19^{a,x}	1.28±0.19^{a,x}	0.78±0.15^{b,x}
72	49.14±2.92^{a,x}	74.16±0.760^{b,x}	48.84±13.40^{a,x}	1.54±0.20^{a,x}	1.00±0.16^{a,x}	1.92±1.46^{a,x}
96	49.60±2.61^{a,x}	77.54±16.93^{b,x}	55.96±13.41^{a,x}	1.44±0.15^{a,x}	0.98±0.17^{a,x}	1.23±0.69^{a,x}
120	49.10±2.36^{a,x}	64.24±10.57^{b,x}	41.54±9.560^{a,x}	1.46±0.19^{a,x}	0.97±0.15^{b,x}	0.79±0.10^{b,x}
144	49.74±2.03^{a,x}	53.36±1.360^{a,x}	43.20±7.850^{a,x}	1.52±0.21^{a,x}	0.99±0.28^{b,x}	1.35±0.29^{a,x}
168	48.78±3.84^{a,x}	53.54±3.600^{a,x}	43.12±5.390^{a,x}	1.54±0.15^{a,x}	1.13±0.22^{b,x}	1.35±0.29^{a,x}

^{a,b}Means with different superscripts with in row differed significantly ($p<0.05$) due to treatment effect; ^{x}Means with different superscripts with in column differed significantly ($p<0.05$) due to time effect

Table 14: Calcium (Ca) and Sodium (Na) of challenged and non challenged mice (Mean±SD)

	Parameters					
	Ca (mmol/L)			Na (mmol/L)		
Time (h)	Control	*C. pseudotuberculosis*	Exotoxin (PLD)	Control	*C. pseudotuberculosis*	Exotoxin (PLD)
12	2.49±0.26$^{a, x}$	2.33±0.03$^{a, x}$	3.33±0.45$^{a, x}$	154.50±13.86$^{a, x}$	148.66±2.56$^{a, x}$	160.32±5.740$^{a, x}$
24	2.52±0.26$^{a, x}$	2.50±0.09$^{a, x}$	2.96±0.39$^{a, x}$	154.50±13.86$^{a, x}$	152.76±1.90$^{a, x}$	165.36±7.950$^{a, x}$
48	2.50±0.21$^{a, x}$	2.38±0.27$^{a, x}$	2.50±0.22$^{a, x}$	153.52±14.74$^{a, x}$	148.94±4.88$^{a, x}$	170.70±18.91$^{a, x}$
72	2.49±0.26$^{a, x}$	2.59±0.11$^{a, x}$	2.47±0.26$^{a, x}$	154.50±13.86$^{a, x}$	145.22±8.24$^{a, x}$	170.42±18.94$^{a, x}$
96	2.49±0.26$^{a, x}$	2.73±0.26$^{a, x}$	2.53±1.30$^{a, x}$	154.50±13.86$^{a, x}$	139.62±4.57$^{a, x}$	161.30±55.51$^{a, x}$
120	2.46±0.28$^{a, x}$	2.35±0.29$^{a, x}$	1.87±1.11$^{a, x}$	153.52±14.74$^{a, x}$	147.76±7.77$^{a, x}$	148.16±7.330$^{a, x}$
144	2.49±0.26$^{a, x}$	2.16±0.15$^{a, x}$	2.40±0.24$^{a, x}$	154.50±13.86$^{a, x}$	146.28±5.02$^{a, x}$	159.60±23.20$^{a, x}$
168	2.50±0.25$^{a, x}$	2.22±0.07$^{a, x}$	2.32±0.25$^{a, x}$	154.34±13.65$^{a, x}$	148.16±4.45$^{a, x}$	149.00±6.350$^{a, x}$

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

Table 15: Potassium (K) and Phosphate (Phos) of challenged and non challenged mice (Mean±SD)

	Parameters					
	K (mmol/L)			Phos (mmol/L)		
Time (h)	Control	*C. pseudotuberculosis*	Exotoxin (PLD)	Control	*C. pseudotuberculosis*	Exotoxin (PLD)
12	6.88±1.11$^{a, x}$	6.32±0.81$^{a, x}$	15.70±4.200$^{b, y}$	2.63±0.60$^{a, x}$	2.53±0.30$^{a, x}$	9.24±1.28$^{b, y}$
24	6.94±1.07$^{a, x}$	6.26±0.63$^{a, x}$	24.60±8.330$^{b, z}$	2.35±0.30$^{a, x}$	2.18±0.31$^{a, x}$	15.74±8.37$^{b, z}$
48	6.72±1.18$^{a, x}$	18.90±3.05$^{b, y}$	22.62±4.780$^{c, z}$	2.51±0.65$^{a, x}$	4.50±1.63$^{b, y}$	13.76±0.86$^{c, z}$
72	6.88±1.11$^{a, x}$	22.14±2.75$^{b, y}$	31.68±26.23$^{c, z}$	2.51±0.43$^{a, x}$	6.52±0.64$^{b, z}$	10.68±5.14$^{c, y}$
96	6.88±1.21$^{a, x}$	16.96±8.71$^{b, y}$	15.16±12.26$^{c, y}$	2.63±0.60$^{a, x}$	7.00±1.03$^{b, z}$	6.21±3.26$^{c, y}$
120	6.88±1.11$^{a, x}$	24.46±6.35$^{b, y}$	5.28±0.750$^{a, x}$	2.63±0.60$^{a, x}$	5.13±1.90$^{b, z}$	2.48±0.10$^{a, x}$
144	6.42±0.66$^{a, x}$	9.14±1.72$^{a, x}$	8.46±2.620$^{a, x}$	2.35±0.30$^{a, x}$	5.49±0.56$^{b, z}$	3.52±1.15$^{a, x}$
168	6.88±1.11$^{a, x}$	7.46±1.31$^{a, x}$	7.82±1.070$^{a, x}$	2.60±0.60$^{a, x}$	2.30±0.32$^{a, x}$	3.35±1.20$^{a, x}$

$^{a-c}$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 time effect

Table 16: Chloride (Cl) of challenged and non challenged mice (Mean±SD)

	Parameters (Cl (mmol/L))		
Time (h)	Control	*C. pseudotuberculosis*	Exotoxin (PLD)
12	103.74±4.67$^{a, x}$	106.66±4.12$^{a, x}$	116.64±4.590$^{a, x}$
24	104.50±5.44$^{a, x}$	110.08±2.90$^{a, x}$	114.60±0.590$^{a, x}$
48	103.74±4.67$^{a, x}$	103.78±7.72$^{a, x}$	118.24±1.890$^{a, x}$
72	103.74±4.67$^{a, x}$	100.90±7.53$^{a, x}$	119.10±25.32$^{a, x}$
96	103.88±7.96$^{a, x}$	90.64±7.47$^{a, x}$	108.18±34.42$^{a, x}$
120	103.74±4.67$^{a, x}$	103.22±9.76$^{a, x}$	95.68±6.820$^{a, x}$
144	103.48±4.90$^{a, x}$	106.64±8.92$^{a, x}$	103.78±16.41$^{a, x}$
168	103.34±2.76$^{a, x}$	104.26±7.67$^{a, x}$	100.70±13.37$^{a, x}$

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

the values. The rise in globulins, noticed in both infected group could reflect the important role played by this protein in the formation of α or β antibodies. Since, serum albumin was drastically decreased in challenged groups at some stages whereas globulins increased, so total proteins in this study were not significantly changed.

CONCLUSION

A critical mass of knowledge with accurate reliability in haemato-biochemical values of CLA has been required over recent years. Therefore, the study indicated for the first time that manipulation of *C. pseudotuberculosis* and its extracted exotoxin (PLD) in experimenatal animals (mouse model) led to haematological and biochemical alterations that were similar to those conducted on small ruminant research of experimentally nature. It is accepted that mice is a suitable model to study the pathogenesis of CLA. Whole bacterium and exotoxin (PLD) intervention are considered important components for vaccine production and prevention strategy for casous lymphadenitis. The present study has documented for the first time several abnormalities in almost all haematologically and biochemically investigated parameters and has pointed to the significance of this information in both establishing base line data and to control these abnormalities for future clinical settings. Thus, it is strongly recommended to have detailed study of CLA, in an attempt to produce treatment or effective vaccine for this disease. Overall, this study has succeeded in fulfilling the objectives of the assigned parameters with respect to haematological levels and biochemistry profiling via manipulation both whole bacterium and exotoxin in mice modelled experimental studies.

ACKNOWLEDGEMENTS

The researchers would like thank Prof. Dr. Peter David Eckersall, University of Glasgow,Veterinary School, for his valuable contribution. Researchers also thank Mr. Yap Keng Chee and Mr. Mohd Jefri Bin Norsidin for their technical assistance. This research was funded under the Research University Grant Scheme (RUGS), Escience Fund and Ministry of Science, Technology and Innovation (MOSTI).

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