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Original Research Articles

Clinio-Pathological Changes in Goats Challenged with *Corynebacterium Peudotuberculosis* and its Exotoxin (PLD)

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Abstract: Caseous lymphadenitis (CLA) is a chronic disease caused by *Corynebacterium pseudotuberculosis*. However, there is a paucity of data about the *C. pseudotuberculosis* exotoxin, phospholipase D (PLD) response during the course of CLA. Therefore, this study was conducted to observe the clinical signs and the cellular changes after an experimental infection of the *C. pseudotuberculosis* and phospholipase D challenge. Twenty six crossbred Boer goats aged 12-14 months were divided into 3 groups; the first group n=6 was inoculated with 1ml sterile PBS s.c. as the control. The second group n=10 was inoculated with live *C. pseudotuberculosis* 1×10^9 cfu s.c. The third group n=10 was i.v. inoculated with PLD 1mL/20 kg, BW. Both the *C. pseudotuberculosis* and the PLD treated groups showed a significant increase ($p<0.05$) in body temperature, heart rate, respiratory rate and body score. Pathologically, the *C. pseudotuberculosis* and the PLD treated groups showed a significant cellular changes ($p<0.05$) manifested as edema, congestion, infiltration of inflammatory cells mainly lymphocytes and macrophages, hemorrhage, degeneration and necrosis in the visceral organs including the lungs, heart, liver, spleen, kidneys and lymph nodes. *C. pseudotuberculosis* infected group showed abscessation of the lymph nodes and some of the visceral organs. In contrast, PLD inoculation did not lead to any abscess formation in the lymph nodes neither in the visceral organs. It concluded that the *C. pseudotuberculosis* caused typical CLA disease with short incubation period of two weeks. The PLD inoculation showed little clinical signs and it did not lead to abscesses formation externally neither internally, however, it caused obvious cellular changes in the visceral organs as well as in the lymph nodes. PLD play a key role in CLA development, yet it is impossible to trigger granulomatous lesion without the *C. pseudotuberculosis* being present.

Keywords: *Corynebacterium peudotuberculosis*, Phospholipase D, Caseous lymphadenitis, Crossbred Boer, Goats, Clinical Signs, Cellular Changes

Introduction

Economically, caseous lymphadenitis causes significant losses to the farmers and small ruminant industry by affecting the wool and skin quality, carcasses condemnation and downgrading during meet inspection at the abattoir and has been documented on all continents with different prevalence rates (Paton *et al*., 2005; Paton, 2010; Silva *et al*., 2013).

Corynebacterium pseudotuberculosis is the etiological agent of caseous lymphadenitis (CLA) and lymphadenitis which characterized by abscess formation in almost all organs in sheep and goats and other animal species including human beings respectively. Caseous lymphadenitis has a long incubation period ranging between 25 to 140 days. The slow nature of CLA lesions development makes it chronic and may become lifelong disease; this fact is often overlooked. Classical CLA abscess contains thick necrotic cheese like exudates with colors ranging from whitish-creamy to yellow with greenish tinge (Dorella *et al*., 2006; Join-Lambert *et al*., 2006; Baird and Fontaine, 2007; Gordon, 2012).

The disease has obvious clinical manifestation when the lesions become progressive, most commonly external lesions appear as abscesses in the superficial lymph nodes of the body and less often internal lesions seen in deeply laying lymph nodes and the visceral organs such as mediastinal lymph nodes and the lungs respectively (Brown *et al*., 1986; Valli and Parry, 1993; Fontaine and Baird, 2008; Paton, 2010). After the incubation period, CLA lesions are characterized at first with the cardinal signs of inflammation, primarily, swelling, redness and pain upon touch of the affected part. The latter develops an abscess mostly in a lymph node or across a lymphatic vessel which can rupture to discharge yellow-greenish pus developing into thick cheesy material; this can form an ulcer which may heal spontaneously and temporarily after which fresh abscess can buildup (Radostits *et al*., 2000; Fontaine and Baird, 2008; Paton, 2010; Guimarães *et al*., 2011).

Mostly, *C. pseudotuberculosis* colonized in the regional lymph nodes that drainage the area where about the infection took place and the lesions appear shortly as generalized inflammation. Furthermore, phospholipase D (PLD) an exotoxin produced by *C. pseudotuberculosis* has a crucial role in initiating of the lymphadenitis which starts as micro-abscesses in the lymph node cortex. Shortly, pyogranulomatous reaction starts which contains cellular debris and high populations of neutrophil; days later the neutrophil population starts to lessen and replaced by macrophage and monocyte population. The developed pyogranuloma has a centre of soft and semi-fluid purulent pus surrounded by thicker semi-solid necrotic exudates containing clumps of the bacteria and at this stage mineralization and calcification may occur (Baird and Fontaine, 2007; Paton, 2010). Immediately after the pyogranuloma develops, purulent emboli may detach and travel along the efferent lymphatic vessels, then delivered into the blood stream and ended elsewhere to start a new lesion (Radostits *et al*., 2000).

However, phagocytic cells may also play a role in transporting *C. pseudotuberculosis* beyond the initial site of entry and consequent lesions could appear (Pepin *et al*., 1994). Despite the means of infection spread, the lungs were the most prominent organ in which CLA lesions are found. It manifested as broncopneumonia that may develops to pleuritis causing adhesions to the chest wall, diaphragm or pericardium. Too often, mediastinal and bronchial lymph nodes are involved (Paton *et al*., 2005; Paton, 2010). In mice, gross lesions of scattered necrotic foci were observed 2 days post inoculation with the *C. pseudotuberculosis* in the liver, kidneys, lymph nodes and the spleen. However, the heart, intestine and the brain showed gross lesions, but to lesser extent; the mortality rate reached 17%.

Moreover, intraperitoneal inoculation of PLD in the same study showed severe hemorrhage in all internal organs particularly, intestines where it accompanied by obvious edematous swelling with renal anemic changes; the mortality rate reached 22% within 48hrs of the PLD inoculation and the cause of death was sought to be systemic toxemia. Histopathological examination showed varying degrees of general inflammation signs such as congestion, edema, degeneration, necrosis, infiltration of polymorph nuclear leukocytes and macrophages post inoculation with the *C. pseudotuberculosis* and PLD in mice (Jesse *et al*., 2011). Similarly, lesions such as congestion, hemorrhage, degeneration, necrosis, infiltration of neutrophil and macrophage, with formation of micro-abscesses were observed in the liver and the kidneys. The lungs showed the exact same lesions of the liver and kidneys with increased vascularisation and hemorrhage in the alveolar and bronchioles lumen in mice inoculated with the *C. pseudotuberculosis* orally (Jesse *et al*., 2013). In naturally CLA infected rams, the liver and the kidneys showed degenerative damage, whilst in the lungs microscopic examination revealed congestion, hyperplasia of bronchi epithelium, vasculitis and thrombosis in the pulmonary arterioles (Ibtisam, 2008). Therefore, this study was designed to observe the clinical signs and cellular changes in goats following *C. pseudotuberculosis* infection. In addition, to study the clinical symptoms and the cellular changes that will be manifested due to the PLD challenge to get better understanding of CLA pathogenesis and the means by which CLA affecting various body systems and organs to fill the gap in the PLD research.

Materials and Methods

Isolation and Identification of Corynebacterium Pseudotuberculosis

Bacteria were isolated from clinical cases of caseous lymphadenitis in goats. Isolates were sent to the Veterinary Laboratory Service Unit (VLSU), Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia for identification and confirmation of the bacteria according to principles and methods described in the microbiological diagnostic laboratory at the Veterinary Medical Teaching Hospital, University of California, Davis, Revised Edition 2008.

Extraction of Phospholipase D

C. pseudotuberculosis exotoxin was extracted following the method described by Zaki (1968). Briefly, 2 or 3 loops of a 48hrs culture of *C. pseudotuberculosis* were inoculated into flask of freshly prepared bovine heart-liver medium. The flask was incubated anaerobically for 7 days at 37°C in slanting position of 15° to 20°. The culture that developed a pellicle was used.

Table 1. The lesion scoring system and the examined organs

Organ/Lesion	Oedema	Inflammatory cells	Degeneration and necrosis	Congestion
Lung	√	√	√	√
Heart	√	√	√	√
Liver	√	√	√	√
Spleen	√	√	√	√
Kidney	√	√	√	√
Lymph nodes	√	√	√	

√ = significant lesions observed in the various organs

Phospholipase D separation started with centrifugation of the culture medium at 8000 rpm/15 min. The supernatant was collected and passed via sterile cellulose membrane filter (0.2 µm) and stored at 4°C then used in the experiment.

Experimental Inoculation

Twenty six crossbred Boer goats (13 buck and 13 doe) aged between 12 to 14 months with no history of vaccination against CLA were screened twice (3 months apart) for CLA Using Agar Gel Immune Diffusion test (AGID) prior to the experiment. The experiment was conducted according to the guidelines and approval of the Institutional Animal Care and Use Committee (IACUC) of Universiti Putra Malaysia, UPM (UPM/FPV/PS/3.2.1.551/AUP-R119).

The goats were divided randomly into 3 groups; the 1st group consisted of 6 goats (3 males and 3 females) housed separately and inoculated with 1ml sterile PBS subcutaneously as control. The 2nd group consisted of 10 goats (5 males and 5 females housed separately) was inoculated with live *C. pseudotuberculosis* 1×10^9 cfu subcutaneously; the 3rd group also consisted of 10 goats (5 males and 5 females housed separately) inoculated with PLD 1mL/20 kg body weight intravenously.

Clinical Signs

Clinical signs were monitored on daily basis which includes body temperature, heart rate, respiratory rate, rumen motility and body score. Site of the inoculation and lymph nodes were observed and recorded on daily basis as well. Parotid, mandibular, pre-scapular, pre-femoral, popliteal, supra-mammary and inguinal lymph nodes were monitored in this study.

Post Mortem Examination

Necropsy was carried out on the goats and gross lesions were recorded. The lung, heart, liver, kidney, spleen and lymph nodes especially parotid, submandibular, prescapular, prefemoral, popliteal, supramammary, inguinal, mediastinal and mesenteric lymph nodes were examined and fixed in 10% buffered formalin solution for histopathological examination.

Histopathology

After fixation, all samples were processed using automatic tissue processor then embedded in paraffin wax and cut at 4µm and the tissue sections were placed on glass slides, stained by Hematoxylin and Eosin and cover with a drop of DPX and cover slip (Luna, 1968).

Lesion scoring was carried out to estimate the cellular changes and its severity. Twenty slides of each organ were selected and six microscopic fields were examined under light microscope using 200X magnification. The scoring system consisted of four scores; 0= normal (no apparent lesion), 1= mild (less than one third of the field involves), 2= moderate (up to two thirds of the field was involved) and 3 (if more than two thirds of the field was involved). The lesion scoring system and the examined organs are shown in Table 1.

Statistical Analysis

Statistical analysis was performed using SPSS version 19.0. Repeated Measure Analysis of Variance (MNOVA) was used to analyze the clinical signs. One way Analysis of Variance (ANOVA) was used to analyze the cellular changes severity. All values were reported as mean ± SE at 95% confidence level.

Results

C. pseudotuberculosis infected animals showed various clinical signs, for instance, increased body temperature, heart rate, respiratory rate and changes in body score as well as abscess formation at the site of inoculation was seen discharging pus two weeks post infection (Fig. 1). Nevertheless, animals maintained normal appetite and constant level of food consumption. Some hair loss was noticed at the lumbar region of the back particularly in the bucks, with signs of pica.

Phospholipase D challenged group showed changes in body temperature, heart rate, respiratory rate and body score as well. There were no signs of any lesions at the site of inoculation and appetite was maintained.

Staggering gait and ataxia of hind limbs were observed in some doe (two weeks post inoculation). Lumpy lesions covered with dry dark crust were seen on the ears of one buck only; upon examination it was solid, cold and painless (Fig. 2). Eyes were affected in the PLD challenged bucks only manifested by swelling around the eyes, serous fluid discharge at the beginning then developed into purulent discharge which continued for several days then healed without any treatment (Fig. 3). Rumen motility showed no changes in both challenged groups throughout the experimental period.

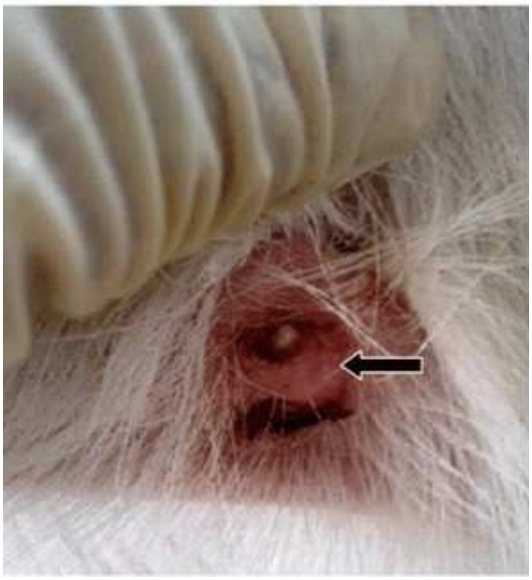

Fig. 1. Inoculation site of the *C. pseudotuberculosis* shows an open abscess with purulent discharge

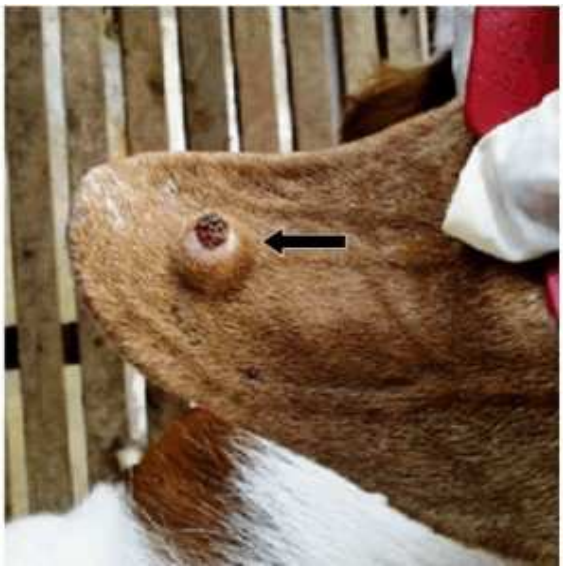

Fig. 2. Lumpy lesion on buck ear inoculated with the PLD

In general, both the *C. pseudotuberculosis* and the PLD inoculation have led to pathological changes in all the visceral organs such as the lungs, heart, liver, spleen, kidneys and the lymph nodes. In addition, the *C. pseudotuberculosis* infection caused abscess formation in the lymph nodes and some visceral organs. In contrast, the PLD inoculation did not cause any abscess formation in the lymph nodes neither in the visceral organs. The histopathological changes manifested as edema, congestion, infiltration of inflammatory cells mainly lymphocyte and macrophage, hemorrhage, degeneration and necrosis.

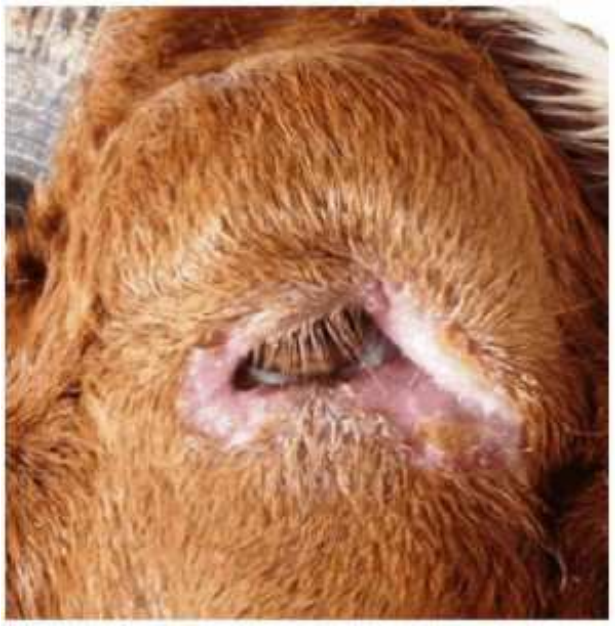

Fig. 3. Eye swelling and purulent discharge in the PLD inoculated bucks

Body Temperature

C. pseudotuberculosis infected group showed a significant rise ($p<0.05$) in body temperature in week 2 (40.0±0.17°C), week 3 (40.6±0.16°C) and week 4 (40.0±0.15°C) then returned to its normal level till end of the experiment compared to the control (39.0±0.20°C), whilst the PLD challenged group showed a significant decrease ($p<0.05$) in body temperature through week 2 to week 6 (38.6±0.10°C, 38.3±0.18°C, 38.6±0.10°C, 38.3±0.18°C and 38.4±0.12°C respectively) when compared with the *C. pseudotuberculosis* inoculated group, but there was no significant difference ($p>0.05$) compared to the control (39.0±0.20°C), (Table 2).

Heart Rate

The heart rate of both the *C. pseudotuberculosis* and the PLD challenged groups was significantly higher ($p<0.05$) than the control (80.66±2.33 bpm) in week 1 (85.00±1.22 bpm, 86.80±2.17 bpm respectively) then it returned to its normal rate between week 2 (84.80±2.74 bpm, 85.00±2.70 bpm) and week 3 (86.60±2.15 bpm, 79.80±1.15 bpm). However, it significantly decreased ($p<0.05$) in both treatment groups in week 4 (80.20±1.46 bpm, 80.20±1.77 bpm respectively) compared to the control (80.66±2.33 bpm), (Table. 3).

Respiratory Rate

The respiratory rate showed no significant ($p>0.05$) changes in both the *C. pseudotuberculosis* and the PLD challenged groups.

Table 2. Temperatur (°C) of the goats post inoculation with *C. pseudotuberculosis* and phospholipase D (Mean ± SE)

Groups weeks	Control °C	*C. pseudotuberculosis* °C	Phospholipase D °C
1	38.8±0.43	38.9±0.15	39.0±0.16
2	38.6±0.16	*40.0±0.17	*38.3±0.10
3	38.4±0.38	*40.6±0.16	*38.3±0.18
4	38.6±0.25	*40.0±0.15	*38.3±0.10
5	39.2±0.26	39.6±0.17	*38.1±0.13
6	39.3±0.23	38.7±0.23	*38.4±0.12
7	39.1±0.08	38.5±0.11	38.5±0.09
8	38.3±0.16	38.5±0.10	38.6±0.17
9	39.4±0.10	38.5±0.09	38.8±0.16
10	39.0±0.20	38.7±0.15	38.7±0.14
11	39.6±0.17	38.9±0.11	38.7±0.13
12	38.9±0.12	39.0±0.19	38.6±0.12

*Significant value p<0.05. Comparison between inoculated groups and the control

Table 3. Heart Rate (bpm) of the goats post inoculation with *C. pseudotuberculosis* and phospholipase D (Mean ± SE)

Groups weeks	Control bpm	*C. pseudotuberculosis* bpm	Phospholipase D bpm
1	78.33±0.88	*85.00±1.22	*86.80±2.17
2	80.66±2.33	84.80±2.74	85.00±2.70
3	83.00±2.88	86.60±2.15	79.80±1.15
4	87.00±1.15	*80.20±1.46	*80.20±1.77
5	84.00±4.04	83.20±2.97	83.20±1.52
6	83.66±2.18	85.40±2.11	84.00±1.92
7	81.00±3.05	82.40±2.37	81.20±1.80
8	77.66±0.33	80.40±2.37	82.60±2.06
9	79.66±2.02	82.40±2.35	81.80±1.62
10	83.00±3.46	80.40±1.93	80.00±1.87
11	79.00±2.51	81.00±2.46	84.40±2.80
12	78.66±1.20	82.80±2.26	83.60±2.46

*Significant value p<0.05. Comparison between inoculated groups and the control

Table 4. Respiratory Rate (bpm) of the goats post inoculation with *C. pseudotuberculosis* and phospholipase D (Mean ± SE)

Groups weeks	Control bpm	*C. pseudotuberculosis* bpm	Phospholipase D bpm
1	28.00±0.88	31.40±1.16	31.60±1.43
2	27.33±1.45	31.00±0.83	29.80±0.73
3	26.33±2.60	*30.60±0.92	*28.80±0.66
4	19.00±3.46	17.60±1.50	18.20±1.71
5	18.00±0.57	*23.40±3.10	*23.40±2.37
6	19.00±1.52	16.40±1.40	20.80±2.35
7	17.33±1.20	18.20±0.37	16.20±0.73
8	17.00±0.57	19.40±1.32	20.80±2.22
9	16.00±1.73	16.80±1.20	16.80±0.80
10	17.66±1.76	19.40±1.83	18.00±1.00
11	20.33±1.45	19.00±1.26	17.20±0.80
12	18.00±1.52	16.60±1.36	17.60±1.07

*Significant value p<0.05. Comparison between inoculated groups and the control

Table 5. Body score of the goats post inoculation with *C. pseudotuberculosis* and phospholipase D (Mean ± SE)

Groups weeks	Control	*C. pseudotuberculosis*	Phospholipase D
1	3±0.35	3±1.35	3±0.0
2	3±0.70	3±1.82	3±0.04
3	3±0.22	3±0.41	3±0.17
4	3±1.01	3±0.22	3±0.25
5	4±0.42	*3±0.33	3±0.12
6	4±0.11	*3±0.34	3±0.02
7	4±0.19	*2±0.18	*4±1.32
8	4±0.23	*2±0.06	*4±0.06
9	4±0.15	*2±0.17	*4±0.21
10	4±0.01	*2±0.02	*3±0.43
11	4±0.0	*2±0.08	*3±0.01
12	4±0.27	*2±1.35	*3±0.13

*Significant value p<0.05. Comparison between inoculated groups and the control

Table 6. Mean score of cellular changes in the lung of goats post inoculation with *C. pseudotuberculosis* and phospholipase D

Parameters	Control	*C. pseudotuberculosis*	Phospholipase D
Oedema	0.00±0.00	*0.19±0.45	*2.22±0.81
Congestion	0.00±0.00	*0.14±0.37	*0.81±0.25
Inflammatory cells	0.00±0.00	*3.22±0.60	*2.07±0.52
Degeneration and necrosis	0.00±0.00	*1.7±0.61	*2.09±0.59

*Significant value $p<0.05$, comparison between inoculated groups and the control

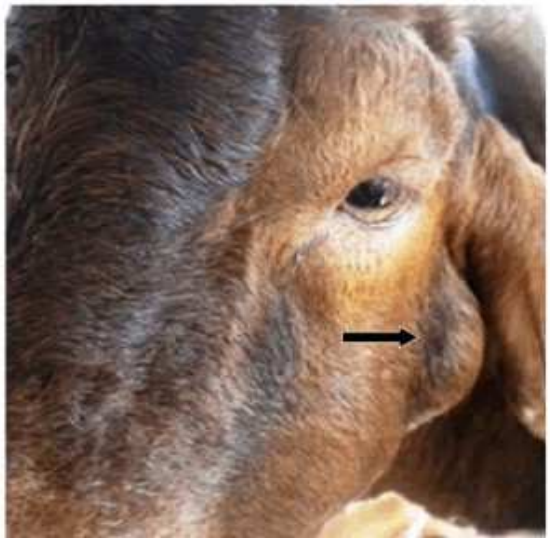

Fig. 4. Affected lymph nodes in the *C. pseudo*tuberculosis infected animals shows enlarged parotid lymph node

However, there was a significant increase ($p<0.05$) in week 3 (30.60±0.92 bpm, 28.80±0.66 bpm respectively) and week 5 (23.40±3.10 bpm, 23.40±2.37 bpm respectively) compared to the control (18.00±0.57 breath/min), (Table 4).

Body Score

Animals in the *C. pseudotuberculosis*, the PLD and the control groups had body score of 3 during the first month of the experiment. *C. pseudotuberculosis* infected group showed two folds significant decrease ($p<0.05$) in body score through week 7 to week 12 (2±0.18, 2±0.06, 2±0.17, 2±0.02, 2±0.08 and 2±1.35) compared to the control (4±0.19). The PLD challenged group showed a significant increase ($p<0.05$) in body score in week 7 (4±1.32), week 8 (4±0.06) and week 9 (4±0.21) compared to the *C. pseudotuberculosis* infected group. However, the PLD challenged group showed a significant decrease ($p<0.05$) in body score in week 10 (3±0.43), week 11 (3±0.01) and week 12 (3±0.13) compared to the control (4±0.19), (Table. 5).

Lymph Nodes

C. pseudotuberculosis infected group showed an enlargement of their lymph nodes compared with other treatment groups. In male, the sequence of lymph nodes enlargement was as follows: Parotid lymph node came first in order, inguinal was second followed by submandibular and prescapular lymph nodes; whilst in female, the sequence was as follows: Submandibular lymph node was the first followed by parotid and prescapular lymph nodes (Fig. 4).

Pathology of the Lung

The lungs from the *C. pseudotuberculosis* infected animals showed no congestion on the surface, but appeared mosaic with varied degrees of red and gray hepatization involving all lobes of the lungs. However, the lungs showed disseminated spots of distinct congestion upon cut (Fig. 5). A deeper cut into the lung parenchyma revealed thick whitish and lumpy pus discharge (Fig. 6).

Microscopic examination of the lungs from *C. pseudotuberculosis* infected animals showed a significant ($p<0.05$) and severe infiltration of inflammatory cells with mean lesion score of 3.22±0.60 compared to the control with mean lesion score of 0.00±0.00 (Table 6). Moreover, there was significant ($p<0.05$) moderate edema with mean score lesion of 0.19±0.45 compared to the control with mean lesion score of 0.00±0.00 (Table 6). The lung tissues also showed moderate and significant ($p<0.05$) congestion with mean score lesion of 1.4±0.37 compared to the control with mean lesion score of 0.00±0.00 (Table 6). The alveolar cells and bronchial epithelium showed a significant ($p<0.05$) moderate degeneration and necrosis with mean lesion score of 1.75±0.61 compared to the control with mean score lesion of 0.00±0.00 (Table 6), (Fig. 7).

The lungs in the PLD inoculated group showed some light and dark spots of gray hepatization (Fig. 8). However, there was no congestion and no abscess formation in the lung parenchyma.

Microscopically, the lung tissues from the PLD challenged animals showed a significant ($p<0.05$) severe edema with mean lesion score of 2.22±0.81 compared to the control with mean lesion score of 0.00±0.00 (Table 6). Congestion also was moderately significant ($p<0.05$) with mean lesion score of 0.18±0.25 compared to the control with mean lesion score of 0.00±0.00 (Table 6). Also there was significant ($p<0.05$) moderate presence of inflammatory cells with mean lesion score of 2.07±0.52 compared to the control with mean lesion score of 0.00±0.00 (Table 6). The alveolar tissue showed a significant ($p<0.05$) severe degeneration and necrosis with lesion mean score of 2.09±0.59 compared to the control with mean lesion score of 0.00±0.00 (Table 6). There was hyperplasia of the bronchiolar epithelium (Fig. 9).

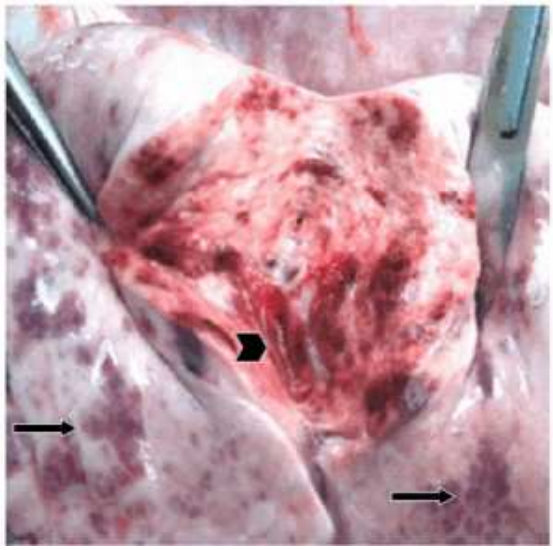

Fig. 5. Lung shows disseminated mosaic discoloration (arrow) and congestion deeper inside (arrow head) post inoculation with the *C. pseudotuberculosis*

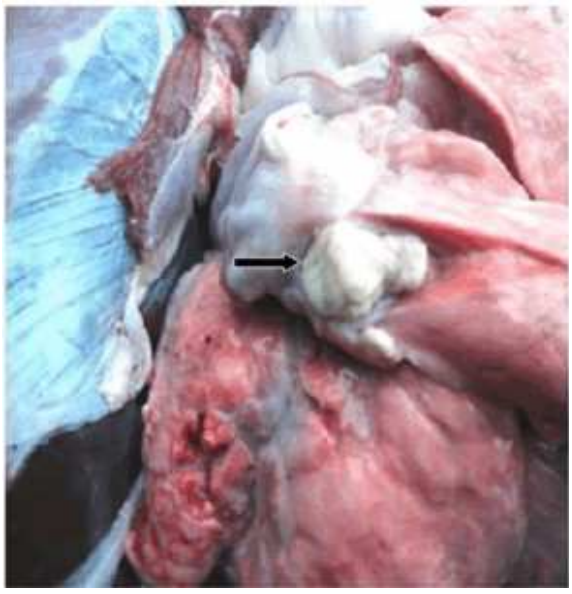

Fig. 6. Lung shows thick whitish and lumpy pus discharge (arrow) post inoculation with the *C. pseudotuberculosis*

Pathology of the Heart

Heart in the *C. pseudotuberculosis* infected goats appeared edematous, pale in color, friable and the pericardium showed some degree of opacity (Fig. 10). Microscopically, the heart tissues from the *C. pseudotuberculosis* infected goats showed mild, but not significant ($p>0.05$) edema with mean score lesion of 0.05±0.25 compared to the control with mean score lesion of 0.02±0.17 (Table 7). Noteworthy, that the heart tissues showed no congestion at all.

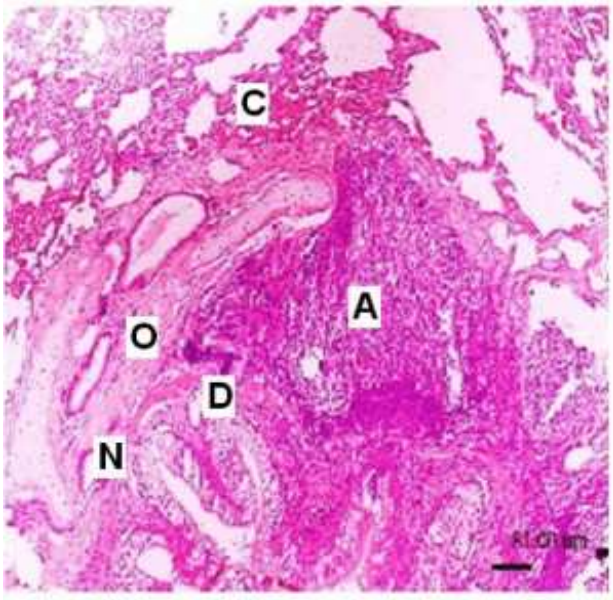

Fig. 7. Lung shows Oedema (O), Congestion (C) microfoci of abscess formation (A), degeneration (D) and Necrosis post inoculation with the *C. pseudotuberculosis*; H&E; 200X

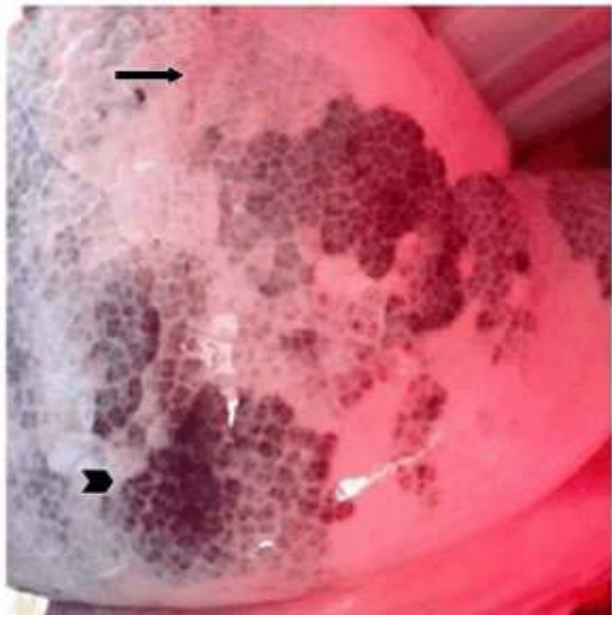

Fig. 8. Lung shows light (arrow) and dark (arrows head) spots of gray hepatization post inoculation with the PLD

The presence of the inflammatory cells was significantly ($p<0.05$) moderate with mean score lesion of 1.98±0.76 compared to the control with mean score lesion of 0.00±0.00 (Table 7). The heart tissues showed a significant ($p<0.05$) moderate degeneration and necrosis with mean score lesion of 2.04±0.49 compared to the control with mean score lesion of 0.00±0.00 (Table 7), (Fig. 11).

Table 7. Mean score of cellular changes in the heart of goats post inoculation with *C. pseudotuberculosis* and phospholipase D

Parameters	Control	*C. pseudotuberculosis*	Phospholipase D
Oedema	0.02±0.00	0.05±0.25	*2.19±0.67
Congestion	0.00±0.00	0.00±0.00	*2.89±0.37
Inflammatory cells	0.00±0.00	*1.98±0.76	*2.01±0.44
Degeneration and necrosis	0.00±0.00	*2.04±0.49	*2.29±0.61

*Significant value p<0.05, comparison between inoculated groups and the control

Table 8. Mean score of cellular changes in the liver of goats post inoculation with *C. pseudotuberculosis* and phospholipase D

Parameters	Control	*C. pseudotuberculosis*	Phospholipase D
Oedema	0.00±0.00	0.23±0.35	*1.69±0.63
Congestion	0.00±0.00	*1.67±0.69	*1.00±0.79
Inflammatory cells	0.00±0.00	*3.06±0.39	0.19±0.11
Degeneration and necrosis	0.00±0.00	*3.13±0.68	*1.88±0.69
Haemorrhage	0.00±0.00	0.00±0.00	*1.17±0.64
Kupffer cells	0.71±0.55	*1.36±0.48	*2.63±0.59

*Significant value p<0.05, comparison between inoculated groups and the control

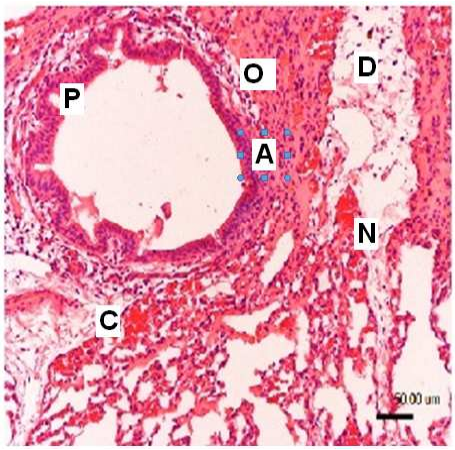

Fig. 9. Lung shows Oedema (O), Congestion (C) infiltration of inflammatory cells (A), Degeneration (D), Necrosis (N) and hyperplasia of bronchiolar epithelium (P) post inoculation with the PLD; H&E; 400X

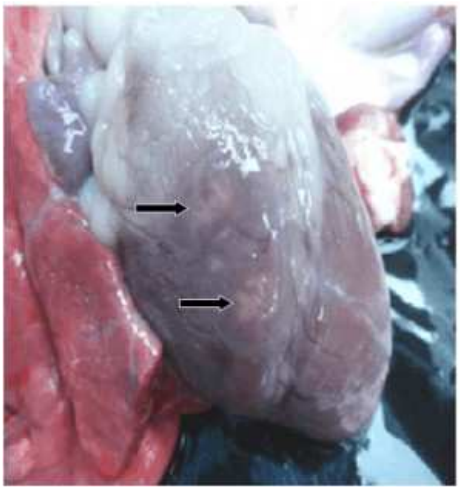

Fig. 10. Heart shows opacity of the pericardium (arrow) post inoculation with the C. *pseudotuberculosis*

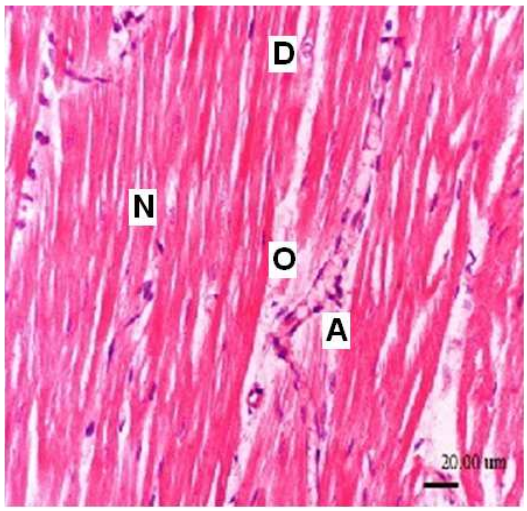

Fig. 11. Heart muscle shows Oedema (O), Degeneration (D), Necrosis (N) and infiltration of inflammatory cells (A) post inoculation with the *C. pseudotuberculosis*; H&E; 400X

The heart from the PLD inoculated group showed no obvious gross changes (Fig. 12). However, microscopic examination revealed significant (p<0.05) moderate edema with mean score lesion of 2.19±0.76 compared to the control with mean score lesion of 0.02±0.17 (Table 7). However, congestion was significantly (p<0.05) severe with mean score lesion of 2.89±0.37 compared to the control with mean score lesion of 0.00±0.00 (Table 7). The presence of the inflammatory cells also was significantly (p<0.05) moderate with mean score lesion of 2.01±0.44 compared to the control with mean score lesion of 0.00±0.00 (Table 7). The heart tissues showed a significant (p<0.05) moderate degeneration and necrosis with mean score lesion of 2.29±0.61 compared to the control with mean score lesion of 0.00±0.00 (Table 7), (Fig. 13).

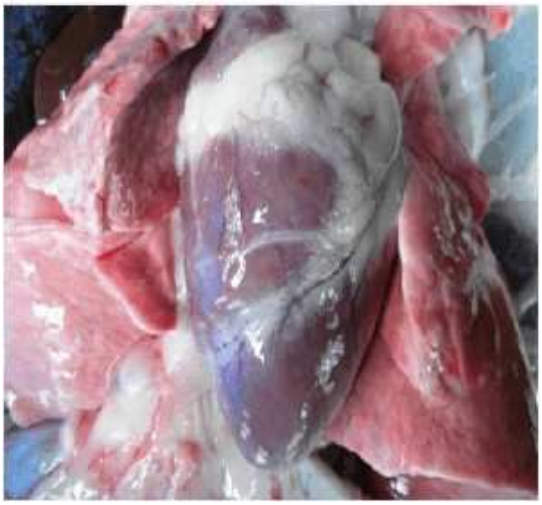

Fig. 12. Heart appeared normal post inoculation with the PLD

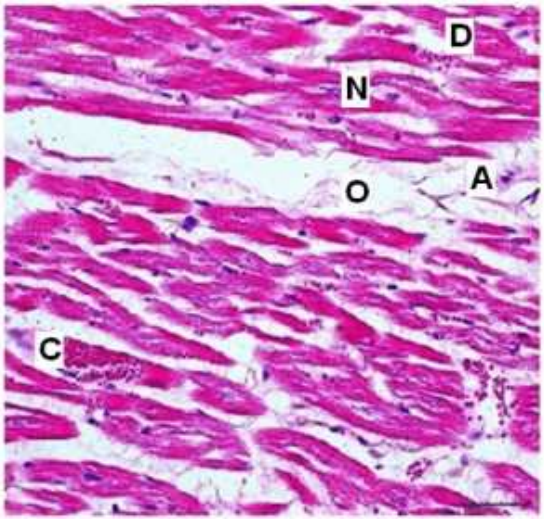

Fig. 13. Heart muscles shows Oedema (O), Congestion (C) Degeneration (D), Necrosis (N) and infiltration of inflammatory cells (A) post inoculation with the PLD; H&E; 400X

Pathology of the Liver

C. pseudotuberculosis infection resulted in multiple abscess formation in the liver parenchyma of the goats. The liver appeared pale and friable with some fibrotic spots surrounding the abscesses (Fig. 14).

Microscopic examination of the liver from the *C. pseudotuberculosis* infected goats showed mild non significant ($p>0.05$) edema with mean score lesion of 0.23±0.35 compared to the control with mean score lesion of 0.00±0.00 (Table 8). The liver tissues showed a significant ($p<0.05$) congestion with mean score lesion of 1.67±0.69 compared to the control with mean score lesion of 0.00±0.00 (Table 8).

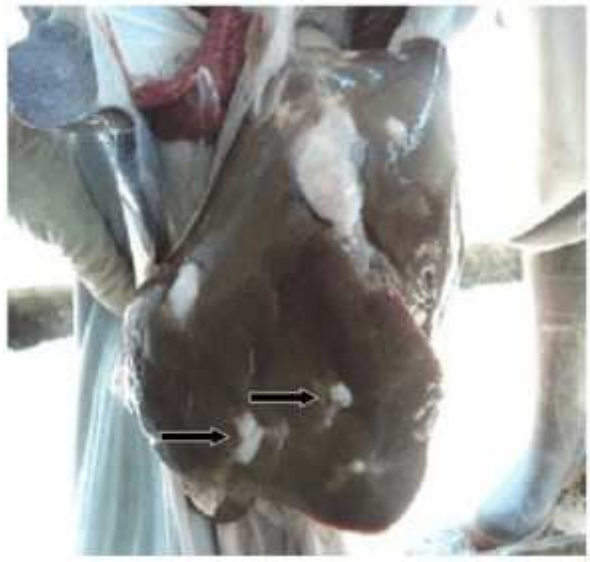

Fig. 14. Liver shows multiple abscess formation (arrow). Notice the fibrosis surrounding the abscesses post inoculation with the *C. pseudotuberculosis*

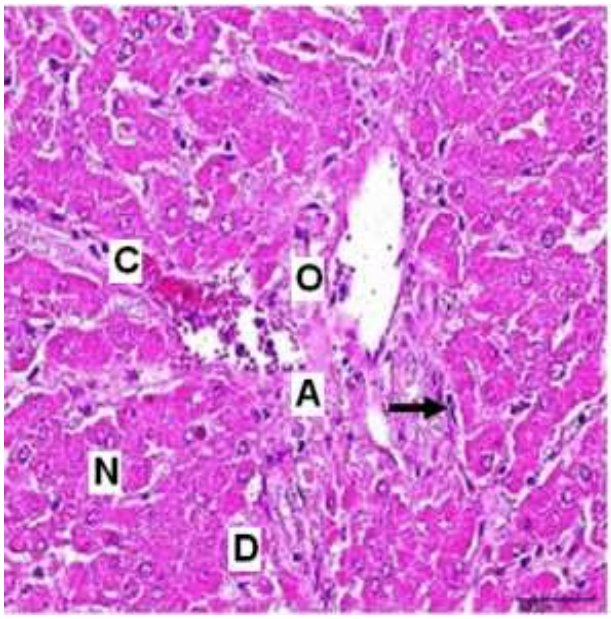

Fig. 15. Liver shows Oedema (O), Congestion (C), infiltration of the inflammatory cells (A), Degeneration (D) necrosis (N) and presence of Kupffer cells (arrow) post inoculation with the *C. pseudotuberculosis*; H&E; 400X

The presence of the inflammatory cells was significantly ($p<0.05$) severe with mean score lesion of 3.06±0.39 compared to the control with mean score lesion of 0.00±0.00 (Table 8). Moreover, degeneration and necrosis of the hepatocytes were significantly ($p<0.05$) severe with mean score lesion of 3.13±0.68 compared to the control with mean score lesion of 0.00±0.00 (Table 8).

Kupffer cells presence was significantly ($p<0.05$) mild with mean score lesion of 1.36±0.48 compared to the control with mean score lesion of 0.71±0.55 (Table 8), (Fig. 15).

The liver from the PLD inoculated animals appeared dark in color and the gall bladder was empty (Fig. 16) with some whitish color line along the edges of the lobe (Fig. 17). Microscopically, the liver tissues from the PLD challenged animals showed a significant ($p<0.05$) moderate edema with mean score lesion of 1.69±0.72 compared to the control with mean score lesion of 0.00±0.00 (Table 8). Also the liver tissues showed a significant ($p<0.05$) mild congestion and hemorrhage with mean score value of 1.00±0.79 and 1.17±0.64 respectively compared to the control with mean score lesion of 0.00±0.00 (Table 8). The presence of the inflammatory cells was not significant ($p>0.05$) with mean score lesion of 0.19±0.11 compared to the control with mean score lesion of 0.00±0.00 (Table 8). Noteworthy, there was depletion of the glycogen from the hepatocytes. Degeneration and necrosis of the hepatocytes were significantly ($p<0.05$) moderate with mean score lesion of 1.88±0.69 compared to the control with mean score lesion of 0.00±0.00 (Table 8). Presence of Kupffer cells was significantly ($p<0.05$) moderate with mean score lesion of 2.63±0.59 compared to the control with mean score lesion of 0.71±0.55 (Table 8), (Fig. 18).

Pathology of the Kidney

The kidney from the *C. pseudotuberculosis* infected group appeared friable and its capsule was opaque (Fig. 19). Microscopically, the renal tissues from the *C. pseudotuberculosis* infected animals showed mild significant ($p<0.05$) edema inside the glomerulus with mean score lesion of 0.90±0.26 compared to the control with mean score lesion of 0.00±0.00 (Table 9). Moreover, the kidney tissues showed mild significant ($p<0.05$) congestion and hemorrhage with mean score lesion of 1.30±0.47 and 0.98±0.33 respectively compared to the control with mean score lesion of 0.00±0.00 (Table 9). Inflammatory cells were present significantly ($p<0.05$) and moderately with mean score lesion of 2.01±0.33 compared to the control with mean score lesion of 0.00±0.00 (Table 9). Severe degeneration and necrosis were significant ($p<0.05$) with mean score lesion of 2.78±0.66 compared to the control with mean score lesion of 0.00±0.00 (Table 9), (Fig. 20). Meanwhile, the kidney from the PLD inoculated animals appeared dark in color, friable and the renal capsule display some opaque spots on its surface (Fig. 21).

Upon microscopic examination, the kidney tissues from the PLD inoculated goats showed moderate significant ($p<0.05$) edema with mean score lesion of 1.62±0.41 compared to the control with mean score lesion of 0.00±0.00 (Table 9). There was mild significant ($p<0.05$) congestion with mean score lesion of 0.88±0.27 compared to the control with mean score lesion of 0.00±0.00 (Table 9). There was scanty non significant ($p>0.05$) infiltration of the inflammatory cells inside the glomeruli with mean score lesion of 0.31±0.73 compared to the control with mean score lesion of 0.00±0.00 (Table 9). Degeneration and necrosis were severely significant ($p<0.05$) with mean score lesion of 3.06±0.54 compared to the control with mean score lesion of 0.00±0.00 (Table 9), (Fig. 22).

Pathology of the Spleen

The spleen from the *C. pseudotuberculosis* infected animals appeared friable and mosaic in color (Fig. 23), whilst the spleen from the PLD inoculated animals appeared friable, the capsule appeared opaque and it revealed dark mosaic discoloration (Fig. 24).

Upon microscopic examination, the spleen tissues from the *C. pseudotuberculosis* infected goats showed sever significant ($p<0.05$) inflammatory reaction and high population of lymphocyte and macrophage aggregations with mean score lesion of 3.14±0.71 compared to the control with mean score lesion of 0.00±0.00 (Table 10). Nevertheless, degeneration and necrosis were severe and significant ($p<0.05$) with mean score lesion of 2.73±0.53 compared to the control with mean score lesion of 0.00±0.00 (Table 10), (Fig. 25).

Whilst in the PLD inoculated animals, the spleen tissues showed mild significant ($p<0.05$) inflammatory reaction and mild population of lymphocyte and macrophage aggregations with mean score lesion of 1.16±0.49 compared to the control with mean score lesion of 0.00±0.00 (Table 10). The edema lesion was very little and non significant ($p>0.05$) with mean score lesion of 0.7±0.34 compared to the control with mean score lesion of 0.00±0.00 (Table 10). There were presence of some spots of degeneration and necrosis of the red pulp with mean score lesion of 1.91±0.66 compared to the control with mean score lesion of 0.00±0.00 (Table 10), (Fig. 26).

Pathology of the Lymph Nodes

In general, the *C. pseudotuberculosis* infected animals developed abscesses in some of the external lymph nodes (parotid, submandibular, prescapular, popliteal) and of some of the internal lymph nodes (mediastinal). Gross examination revealed fluctuant and enlarged lymph nodes which were oozing thick and whitish caseous discharge upon cut (Fig. 27 and 28).

In contrast, the PLD inoculated animals did not develop any abscesses in their lymph nodes neither in other organs. However, the lymph nodes appeared normal externally (Fig. 29), yet have some rusty yellow color inside the lymph node (Fig. 30).

Table 9. Mean score of cellular changes in the kidney of goats post inoculation with *C. pseudotuberculosis* and phospholipase D

Parameters	Control	*C. pseudotuberculosis*	Phospholipase D
Oedema	0.00±0.00	*0.90±0.26	*1.62±0.41
Congestion	0.00±0.00	*1.30±0.47	*0.88±0.27
Inflammatory cells	0.00±0.00	*2.01±0.72	0.31±0.73
Degeneration and necrosis	0.00±0.00	*2.78±0.66	*3.06±0.54
Haemorrhage	0.00±0.00	0.98±0.33	0.00±0.00

*Significant value $p<0.05$, comparison between inoculated groups and the control

Table 10. Mean score of cellular changes in the spleen of goats post inoculation with *C. pseudotuberculosis* and phospholipase D

Parameters	Control	*C. pseudotuberculosis*	Phospholipase D
Oedema	0.00±0.00	0.00±0.00	0.07±0.34
Congestion	0.00±0.00	0.00±0.00	0.00±0.00
Inflammatory cells	0.00±0.00	*3.14±0.71	*1.16±0.49
Degeneration and necrosis	0.00±0.00	*2.73±0.53	*1.91±0.66

*Significant value $p<0.05$, comparison between inoculated groups and the control

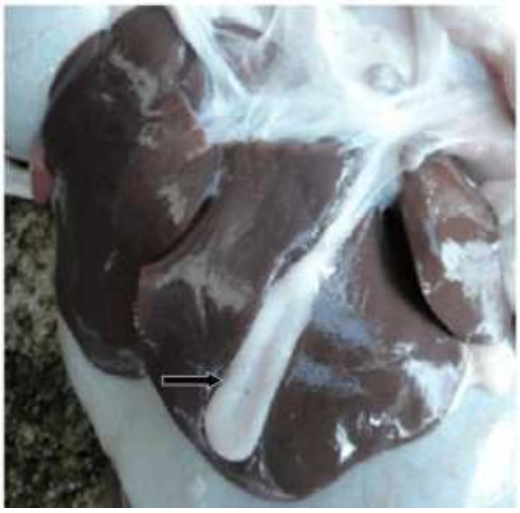

Fig. 16. Liver appeared dark in colour and the gall bladder (arrow) was empty post inoculation with the PLD

Fig. 17. Liver shows whitish colour line (arrow) along the edges of the lobe post inoculation with the PLD

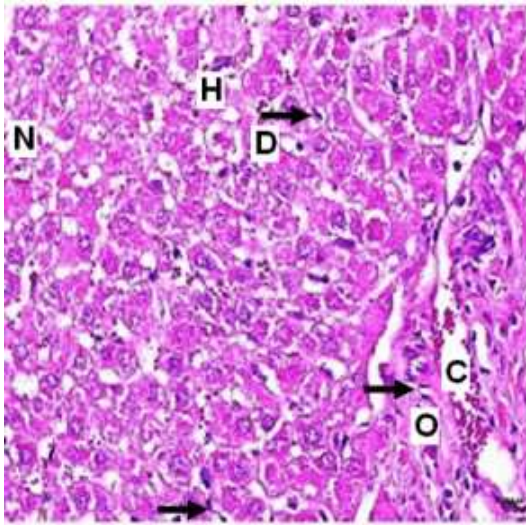

Fig. 18. Liver shows Oedema (O), Congestion (C, mild Haemorrhage (H), Degeneration (D), Necrosis (N) and Kupffer cells (arrow) post inoculation with the PLD; H&E; 400X

Microscopically, lymph nodes from the *C. pseudotuberculosis* infected goats showed a significant ($p<0.05$) moderate edema with mean score lesion of 1.21±0.51 compared to the control with mean score lesion of 0.00±0.00 (Table 11). Moreover, there was significant ($p<0.05$) infiltration of vast numbers of lymphocytes and macrophages marking an area of micro-foci of abscess formation with mean score lesion of 3.25±0.39 compared to the control with mean score lesion of 0.00±0.00 (Table 11), (Fig. 31). The lymph nodes also showed a significant ($p<0.05$) severe hemorrhage with mean score lesion of 2.70±0.17 compared to the control with mean score lesion of 0.00±0.00 (Table 11). Degeneration and necrosis of the lymphatic tissue was significant ($p<0.05$) with mean score lesion of 2.89±0.83 compared to the control with mean score lesion of 0.00±0.00 (Table 11), (Fig. 32).

Table 11. Mean score of cellular changes in the lymph nodes of goats post inoculation with *C. pseudotuberculosis* and phospholipase D

Parameters	Control	*C. pseudotuberculosis*	Phospholipase D
Oedema	0.00±0.00	*1.21±0.51	*1.07±0.48
Congestion	0.00±0.00	0.00±0.00	*0.91±0.69
Inflammatory cells	0.00±0.00	*3.25±0.39	0.00±0.00
Degeneration and necrosis	0.00±0.00	*2.89±0.83	*1.10±0.73
Haemorrhage	0.00±0.00	*2.70±0.17	0.00±0.00
Haemosiderin	0.00±0.00	0.00±0.00	0.25±0.19

*Significant value p<0.05, comparison between inoculated groups and the control

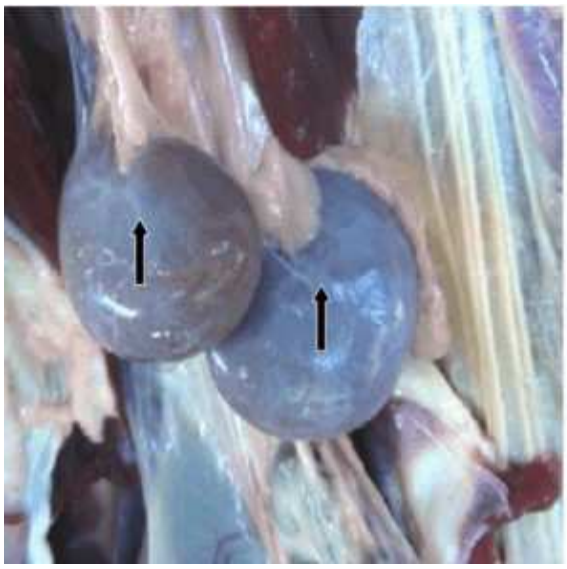

Fig. 19. Kidney shows the opacity of its capsule (arrow), post inoculation with the *C. pseudotuberculosis*

Fig. 21. Kidney shows some opaque spots (arrow) on its surface post inoculation with the PLD

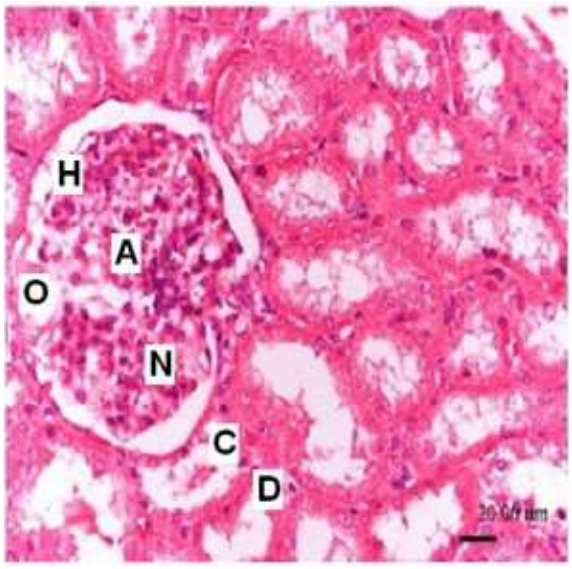

Fig. 20. Kidney shows Oedema (O), Congestion (C, mild Haemorrhage (H), infiltration of inflammatory cells (A), Degeneration (D) and Necrosis (N) post inoculation with the *C. pseudotuberculosis*; H&E; 400X

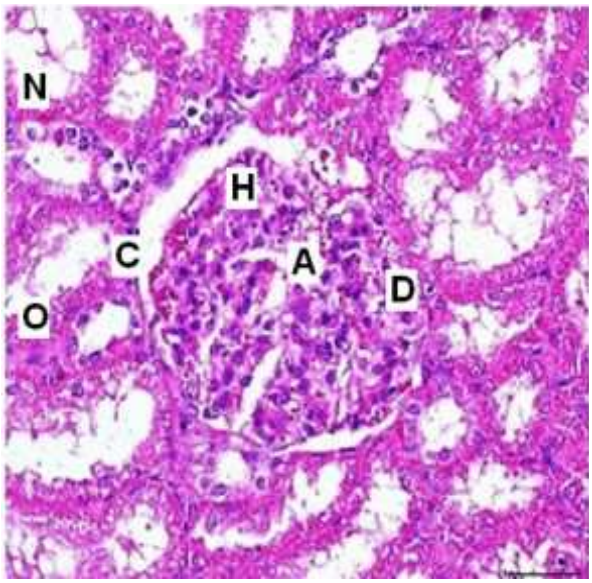

Fig. 22. Kidney shows Oedema (O), Congestion (C, mild Haemorrhage (H), infiltration of inflammatory cells (A), degeneration (D) and Necrosis (N) post inoculation with the PLD; H&E; 400X

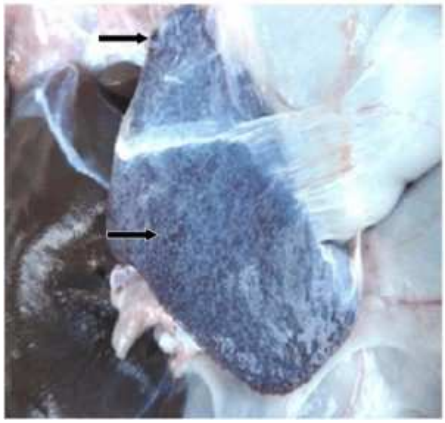

Fig. 23. Spleen shows some mosaic discoloration (arrow) on its surface post inoculation with the *C. pseudotuberculosis*

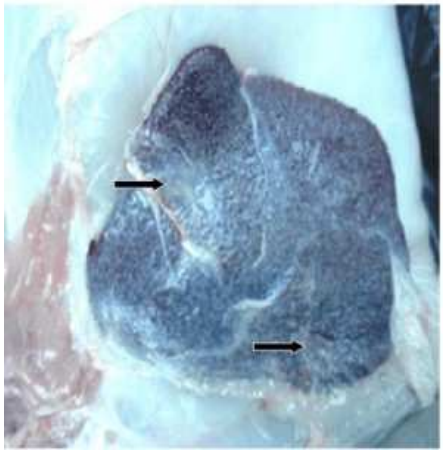

Fig. 24. Spleen shows opaque capsule (arrow) and it revealed dark mosaic discoloration post inoculation with the PLD

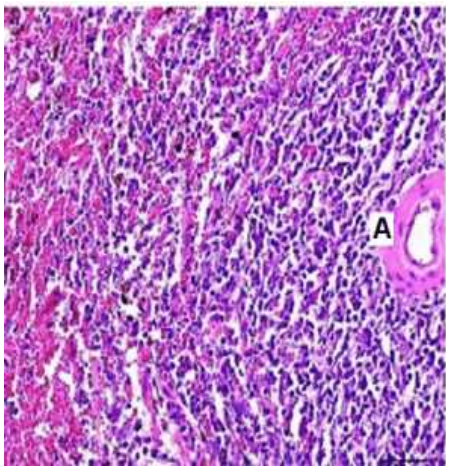

Fig. 25. Spleen shows sever inflammatory reaction with high population of lymphocytes and macrophages aggregation (A) post inoculation with the *C. pseudotuberculosis*; H&E; 400X

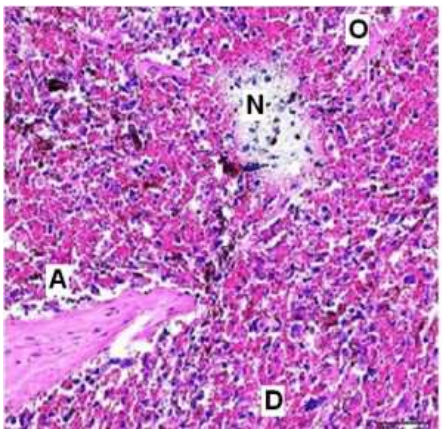

Fig. 26. Spleen shows mild inflammatory reaction with mild population of lymphocytes and macrophages aggregation (A), Oedema (O), Degeneration (D) and Necrosis (N) of some spots of the red pulp post inoculation with the PLD; H&E; 400X

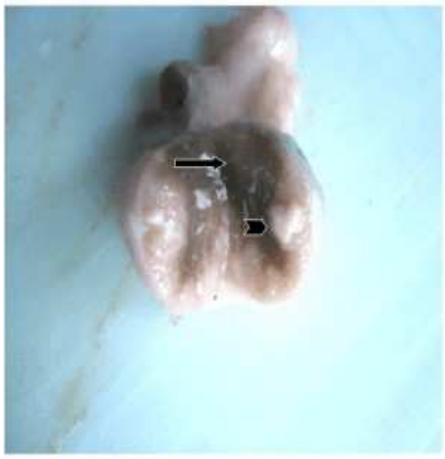

Fig. 27. Lymph node shows congested medulla (arrow) and pus oozing from the cortex area (arrow head) post inoculation with the *C. pseudotuberculosis*

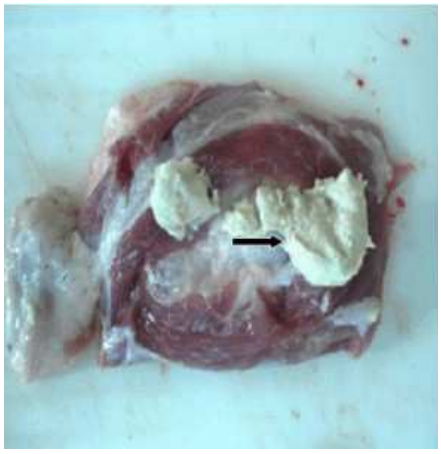

Fig. 28. Lymph node shows thick and whitish caseous discharge (arrow) post inoculation with the *C. pseudotuberculosis*

Fig. 29. Lymph node shows normal appearance (arrow) post inoculation with the PLD

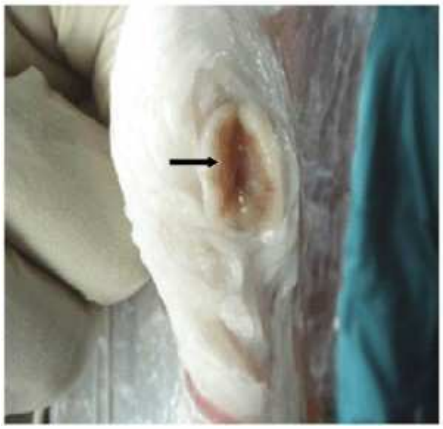

Fig. 30. Lymph node shows some rusty yellow colour inside the lymph node (arrow) post inoculation with PLD

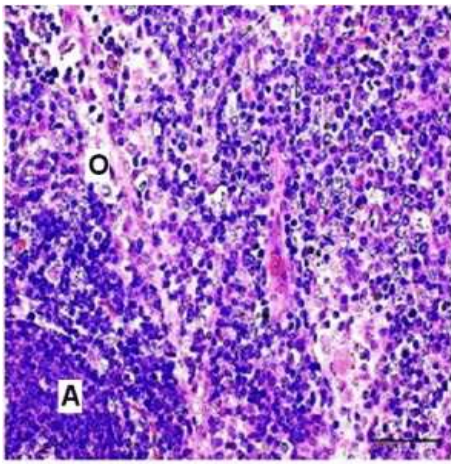

Fig. 31. Lymph node shows Oedema (O), infiltration of vast numbers of lymphocytes and macrophages marking an area of microfoci of abscess formation (A) post inoculation with the *C. pseudotuberculosis*; H&E; 400X

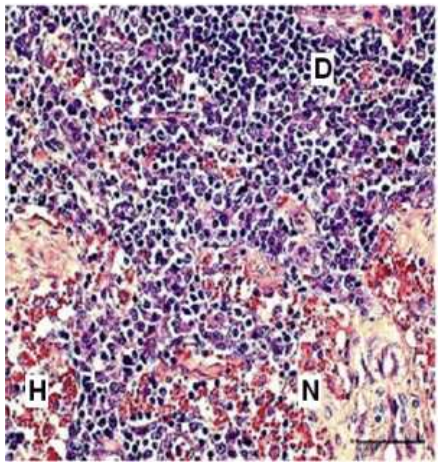

Fig. 32. Lymph node shows Haemorrhage (H), Degeneration (D) and Necrosis (N) of the lymphatic tissue post inoculation with the *C. pseudotuberculosis*; H&E; 400X

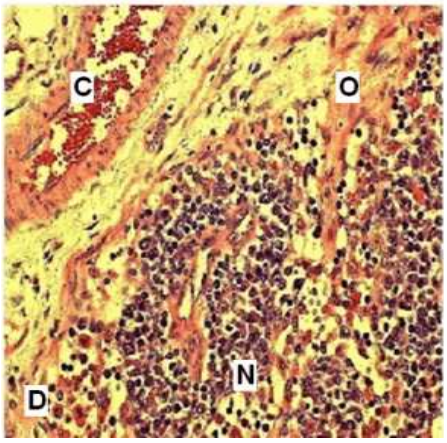

Fig. 33. Lymph node shows Oedema (O), Congestion (C), Degeneration (D) and Necrosis (N) of the lymphatic tissue post inoculation with the PLD; H&E; 400X

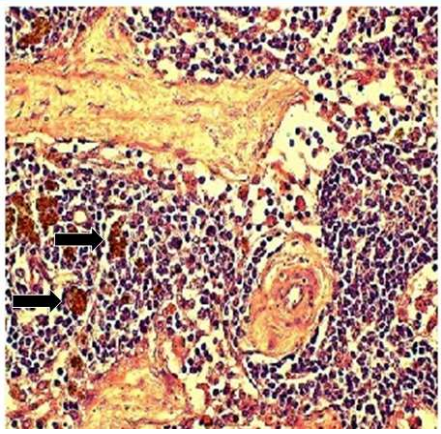

Fig. 34. Lymph node shows scattered haemosiderin (arrow) deposits in the lymphatic tissue post inoculation with PLD; H&E; 400X

In PLD inoculated animals, the lymph nodes showed a significant ($p<0.05$) moderate edema with mean score lesion of 1.07±0.48 compared to the control with mean score lesion of 0.00±0.00 (Table 11). Moreover, lymph nodes showed a mild significant ($p<0.05$) congestion with mean score lesion of 0.91±0.69 compared to the control with mean score lesion of 0.00±0.00 (Table 11). Often, the lymphatic tissues showed a significant ($p<0.05$) degeneration and necrosis with mean score lesion of 1.10±0.73 compared to the control with mean score lesion of 0.00±0.00 (Table 11), (Fig. 33). It's noteworthy to mention there was a scattered non-significant ($p>0.05$) hemosiderin deposits in the lymphatic tissue with mean score lesion of 0.25±0.19 compared to the control with mean score lesion of 0.00±0.00 (Table 11), (Fig. 34).

Discussion

Caseous lymphadenitis is a chronic reoccurring disease that has extended incubation period during which infected animals may not demonstrate any clinical signs until the lesion become progressive and large enough to be noticed. Hence, only external lesions that are sufficiently big or influence the ordinary function of a limb, organ or tissue are noticed during clinical inspection (Paton *et al*., 1996; Paton, 2010).

C. pseudotuberculosis infected group showed significant febrile reaction leading to increase in body temperature. Notably, abscess started to form at the site of injection two weeks post inoculation of the *C. pseudotuberculosis*. At some stage of any infection, fever is the most recurrent clinical sign when microbes invade a host's tissues leading to stimulation of the immune system, most particularly leukocyte which in response produces pro-inflammatory cytokine that induce the production of prostaglandins and increases the body temperature above the set point and produces fever (Dinarello, 1999; Netea *et al*., 2000). The findings from this study is in agreement with Junior *et al*. (2006) who stated that goats challenged with the *C. pseudotuberculosis* developed fever 4 days post inoculation then the body temperature returned to its normal level on day 5. The findings also in agreement with Braga *et al*. (2007) who reported that severe reaction was observed in naïve alpacas two weeks post inoculation with high dose (9×10^8 cfu) of the *C. pseudotuberculosis* and it was manifested by fever and eventually led to the death of the animal.

Inoculation of lipopolysaccharide in rats causes no febrile reaction, but rather leads to decrease in body temperature (Hedger and Hales, 2006). Aberrantly, inoculation with the PLD showed no changes in goat's body temperature. This is contrary to the fact that any exogenous stimulus signified by bacteria itself, bacterial products, live and killed gram positive or gram negative bacteria and toxins initiate fever when inoculated into an animals (Netea *et al*., 2000). The nil effect of the PLD on body's temperature of inoculated animals can be explained in two hypotheses. Firstly: The PLD requires activation by hormones, growth factors or neurotransmitters to exert complete biological activities and implication in immune response and inflammation (Parmentier *et al*., 2006). Secondly: Due to insufficient single dose that has been administered to the animals in the current study as sometimes repeated dosing may also be required.

Both the *C. pseudotuberculosis* and the PLD challenged groups showed a significant rise in heart rate and respiratory rate post inoculations. These results are in accord with the study that conducted by Junior *et al.* (2006) who reported that goats inoculated with the *C. pseudotuberculosis* infected materials showed critical changes such as increasing the heart and the respiratory rates causing tachycardia and tachypnea 2 days post inoculation. Caseous lymphadenitis is a subclinical disease with few specific clinical signs and infected animals exhibite respiratory signs and emaciation (Kaba *et al*., 2011). Oddly, inoculation of the PLD can cause dyspnea (Paton, 2010).

Intradermal inoculation of the *C. pseudotuberculosis* in goats caused papules 2 days post inoculation then transformed to pustules on day 5 which developed into an ulcer covered with dried up crust (Junior *et al*., 2006). Two weeks post inoculation all male and female goats infected with the *C. pseudotuberculosis* showed abscess formation at the inoculation site. The abscess had more than one opening and discharged yellowish creamy pus. These results accords with those reported by Pepin *et al*. (1988) and Pepin *et al*. (1994) who stated that subcutaneous inoculation of different concentrations of the *C. pseudotuberculosis* in sheep resulted in confined reaction at the inoculation site and several abscess formations in the lymph nodes 21 days post inoculation. It also accords with those finding of Selim *et al*. (2010) who reported that subcutaneous inoculation of the *C. pseudotuberculosis* in sheep showed abscess formation at the site of inoculation as well as into prescapular, prefemoral, popliteal, inguinal, bronchio-thorasic and mesenteric lymph nodes.

Body score is a numerical system used to evaluate and assess body weight. Caseous lymphadenitis affecting superficial lymph nodes and/or the visceral organs leads to body mass loss and reduces growth of the body with prominent chronic weight loss (Pugh, 1997; 2002; Gordon, 2012). In the current study goats that were infected with the *C. pseudotuberculosis* and the PLD showed a significant decrease in body weight during different times of the experiment. These results in agreement with those results reported by Junior *et al.* (2006) who stated that goats inoculated with the *C. pseudotuberculosis* infected substances showed inappetence and reduced food intake

3hrs post inoculation and complete stop of food consumption after 48 hrs. Moreover, strains of the *C. pseudotuberculosis* with deleted *pld* gene were incapable of producing persistent infection (Hodgson *et al*., 1990). Phospholipase D, can enhance bacterial invasion by increasing the vascular permeability and causing haemolysis; upon intravenous or subcutaneous inoculation, the PLD can cause dyspnea, icterus and haemoglobinuria in small ruminants (Paton, 2010). Objectively, all the latter activities of the PLD can cause indirect weight loss through diminution of appetite, production of fever and failure of ambulation (Peterhans *et al*., 2004).

In the current study the *C. pseudotuberculosis* infected animals showed lymph nodes enlargement in various parts of the body, mainly, head region. These findings are in agreement with those reported by Unanian *et al*. (1985) who stated that the majority of CLA lesions in goats were in the anterior half of the body in front of the prescapular region then the inguinal region was the other prominent area. Zavoshti *et al*. (2012) conducted a study in Khosroshahr (suburb of Tabriz, East Azerbaijan province, Iran) abattoir on sheep carcasses, the study showed that CLA lesions were mainly found in prescapular, prefemoral, inguinal, supramammary and mediastinal lymph nodes. Conspicuously, the PLD challenged animals showed absolute nil of the abscess formation in any of the lymph nodes and neither in the visceral organs.

This study found that all the *C. pseudotuberculosis* infected goats have developed abscesses in some of their lymph nodes and some of their visceral organs, a result that is consistent with those of Brown *et al*. (1986), Valli and Parry (1993), Radostits *et al*. (2000), Paton *et al*. (2005), Dorella *et al*. (2006), Baird and Fontaine (2007), Fontaine and Baird (2008), Ibtisam (2008), Paton (2010), Guimarães *et al*. (2011), Jesse *et al*. (2011; 2013). In contrast, the PLD inoculated goats showed no abscess formation. However, many cellular changes were detected in some of the lymph nodes as well as the visceral organs. These results are with agreement of Prescott and Muckle (1986) and Paton (2010) that reported that inoculation of the *C. pseudotuberculosis* exotoxin, (PLD), resulted in icterus, dyspnoea, anemia, haemoglobinuria in sheep and goats. Rigorously, the PLD causes dermonecrosis (Muckle and Gyles, 1986), death (Brogden and Engen, 1990), limits bacterial opsonisation and activates the complements pathway (Yozwiak and Songer, 1993).

In the current study, the lungs and its associated thoracic lymphatic's, mainly, mediastinal lymph nodes from the *C. pseudotuberculosis* infected goats appeared congested with many spots of gray and red hepatization. Abscesses were found deep in the lung parenchyma, upon cut thick whitish caseous discharge was revealed. Interestingly, the mediastinal lymph nodes were moderately enlarged and fluctuant, upon cut it revealed even thicker whitish caseous discharge same as the lungs. These results disagree with Paton (2010) stating that CLA lung lesions are uncommon in goats, yet, Baird and Fontaine (2007) stated that in sheep, the internal lesions of CLA are mainly found in the lung's parenchyma and mediastinal lymph nodes. Moreover, Ibtisam (2008) reported severe generalized congestion of the lungs along with abscess formation in rams naturally infected with CLA. Microscopic examination revealed pulmonary congestion, interstitial pneumonia, vasculitis, thrombosis of the pulmonary arterioles, hyperplasia of bronchial epithelia. Additionally, the same previous study reported abscess formation in the pulmonary parenchyma represented by center of caseous necrosis surround by an area of dead and live neutrophil.

Similarly, microscopic examination in our study showed a moderate pulmonary congestion and edema. Feasible degeneration and necrosis also detected in alveolar cells and the lining epithelium of the bronchioles. The most prominent microscopic features were the necrotic foci of caseous necrosis surround by a marked zone of lymphocyte and macrophage representing the micro-abscess formation. The results of this study were consistent with those reported by Valli and Parry (1993) who stated the classical presentation of the histopathological feature of the *C. pseudotuberculosis* infection in sheep is formation of pyogranulomas. Jesse *et al*. (2011) described that the pulmonary lesions in mice model of CLA were congestion and increased vascularisation. Moreover, the lungs showed congestion, hemorrhage and development of micro-abscesses, caseous necrosis and presence of tubercular granuloma that surrounded with neutrophil, macrophage and giant multinucleated cells in mice model of CLA (Osman *et al*., 2012).

To the knowledge of the author, the present study is the first to describe the histopathological changes of the visceral organs post inoculation with the PLD in goats. Osman *et al.* (2012) reported no abscess formation in the visceral organs nor the abdominal lymph nodes in mice inoculated with the PLD via intraperitoneal route. The authors suggested that the formation of the micro-abscesses completely relay on the presence of the pathogen, namely *C. pseudotuberculosis*. Similarly, the lung tissues from the PLD inoculated goats in this study showed a severe edema, moderate congestion and moderate presence of inflammatory cells. On the other hand, the alveolar cells revealed severe degeneration and necrosis with hyperplasia of the bronchiolar epithelium. However, there was nil abscess formation in the pulmonary tissues from the PLD inoculated goats. This disagrees with the previous findings of incriminating the PLD as possible initiator of lymphadenitis (Baird and Fontaine, 2007). Further, Osman *et al*. (2012) mentioned that the histopathological changes of the visceral organs mainly, the lungs, heart, liver, kidney, spleen and the

abdominal lymph nodes from the mice inoculated with the PLD via intraperitoneal route were same as those histopathological changes from the *C. pseudotuberculosis* inoculated mice, but milder and there was no micro-foci of abscess formation.

C. pseudotuberculosis infection in mice caused cellular changes in heart tissues represented by extensive hemorrhage, infiltration of inflammatory cells, degeneration and presence of micro-foci of caseous necrosis (Osman *et al*., 2012). Similarly, in this study, the heart tissues from the *C. pseudotuberculosis* infected goats showed mild edema, but there were no congestion. However, presence of inflammatory cells was significant and cardiac myofibers exhibit severe degeneration and necrosis. These results also with agreement with those results of Ibtisam (2008) who reported that the gross examination of the ram's carcasses naturally infected with CLA revealed severely congested heart. The histopathological changes in the heart tissues were hemorrhage accompanied with hemolysed blood cells. Moreover, infiltration of polymorph nucleated leukocytes was seen in cardiac tissues with degeneration and necrosis of the myofibers.

In mice, the PLD inoculation resulted in generalized edema and hemorrhage in all visceral organs including the heart. Similarly, cellular changes were severe hemorrhage accompanied with infiltration of polymorph nuclear leukocytes (Osman *et al*., 2012). The findings in our study orchestrate with the previous study of Osman *et al*. (2012). The heart tissues from the PLD inoculated goats showed moderat oedema, severe congestion, presence of inflammatory cells, degeneration and necrosis. Phospholipase D, has the ability to hydrolyses the cell membranes increasing the vascular permeability thus, leakage of plasma proteins into the surrounding tissue (Guimarães *et al*., 2011). Moreover, the PLD has lethal effect on living cells and it has been reported to cause dermonecrosis (Tashjian and Campbell, 1983; Brogden and Engen, 1990). Objectively, we theorize that the histopathological changes in the heart tissues are due to the direct toxic effect of the PLD on the myocytes. Moreover, the PLD has led to degeneration and necrosis of the myofibers of the heart.

The liver from the *C. pseudotuberculosis* infected goats revealed multiple abscesses in its parenchyma. Our study findings are with agreement of the previous studies of Davis (1990), Radostits *et al*. (2007) and Paton (2010), all reported that the *C. pseudotuberculosis* infection in sheep can be disseminated beyond the regional or associated lymph nodes to infect the visceral organs and causes abscess formation. Occasionally, those abscesses can be found in the liver, kidneys, heart, spleen and the lungs. Caseous lymphadenitis lesions in these organs are similar to those of the lymph nodes, most likely encapsulated abscesses containing thick cheese like necrotic materials (Valli and Parry, 1993). Microscopically, the liver tissues from the *C. pseudotuberculosis* infected animals showed edema, significant congestion and severe infiltration of inflammatory cells especially lymphocyte and macrophage surrounding a marked area of caseous necrosis. Moreover, there was significant degeneration and necrosis of the hepatocytes. The current study findings are with agreement with those of Ibtisam (2008) who reported severe congestion, hyperplasia of bile ductules, varied degrees of degeneration and necrosis of the hepatocytes of rams naturally infected with CLA. In mice, the *C. pseudotuberculosis* infection causes enlarged and congested liver, presence of bronze discoloration and necrotic foci (mulberry appearance) on the surface. Upon microscopic examination, the liver showed severe congestion with focal zones of abscess formation (Osman *et al*., 2012). However, this study reported that the *C. pseudotuberculosis* infection resulted in significant mild increase in Kupffer cells, a finding added to the new knowledge of CLA in goats.

Grossly, the liver from the PLD inoculated goats appeared dark in color with distinct white line on the edges of the lobes. However, histopathological changes were edema, congestion and hemorrhage. Moreover, the presence of the inflammatory cells, yet hepatocytes manifested significant degeneration and necrosis. In mice, inoculation with the PLD causes severe congestion of the liver. Similarly, Osman *et al*. (2012) stated that histopathological changes in the mice liver were spread hemorrhage, severe congestion and early stage of hepatocytes degeneration. Phospholipase D acts directly on cell membranes weakening it and make it prone to lyses (Titball, 1993, Songer, 1997). It believes that the PLD has had the same effect on the hepatic cells membrane compromising its integrity and resulting in degeneration and necrosis. Hence, the PLD hydrolyses the mammalian cell membrane especially endothelial layer of vasculatures leading to congestion and/or hemorrhage (Guimarães *et al*., 2011). This study reports for the first time that the PLD challenged group showed significant presence of Kupffer cells in the liver tissue. We believe that the increased number of Kupffer cells may be stimulated by the PLD.

The kidneys from sheep naturally infected with CLA were severely congested. However, the histopathological examination showed congestion, hemorrhage and in some cases there was thrombosis. Degeneration and necrosis of the tubular epithelium were obvious along with interstitial infiltration of mononuclear inflammatory cells (Ibtisam, 2008). Indeed, in this study, the kidney tissues from the *C. pseudotuberculosis* infected goats edema, congestion and hemorrhage inside the glomeruli.

There was infiltration of inflammatory cells with degeneration and necrosis of the lining epithelia of the renal tubules. The kidneys from mice infected with the *C. pseudotuberculosis* appeared anemic, friable with macro-abscesses on its surface. The cellular changes were severe degeneration renal tubular epithelium with scattered foci of abscess formation (Osman *et al*., 2012).

In mice, inoculation with the PLD caused severe hemorrhage with edema in the kidneys. Nevertheless, cellular changes were observed are severe congestion, diffuse hemorrhage and infiltration of vast numbers of inflammatory cells mostly neutrophil (Osman *et al*., 2012). In this study, post mortem examination of the kidneys from the PLD inoculated goats revealed dark discoloration of the kidney with some opaque spots on the surface of the renal capsule. However, histopathological examination edema, congestion and there was scanty presence of the inflammatory cells. In fact, the renal tissues showed severe and significant degeneration and necrosis of the lining epithelia of renal tubules. The fact that protein and lipids that compose the mammalian cell membranes make it a major target for bacterial invasion of the host tissues. Some of those bacteria have developed unique invasive toxins such as phospholipase enzyme to hydrolyse phospholipids. The end result of this process is partial or complete damage of the cell membrane compromising its function (Slayer and Witt, 1994; Ghannoum, 2000; Baird and Fontaine, 2007). Similarly, it believes that PLD from the *C. pseudotuberculosis* has caused degeneration and/or necrosis of the host's cells resulting in varied degrees of clinical symptoms.

Osman *et al*. (2012) reported enlarged spleen with multiple necrotic foci on its surface. In addition, the cellular changes were abscess formation and infiltration of polymorph nuclear leukocytes in mice model of CLA. In the current study, the spleen from the *C. pseudotuberculosis* infected goats showed a significant presence of the inflammatory cells, mainly lymphocyte and macrophage surrounding an area of micro-abscesses. Additionally, there was significant degeneration and necrosis of the spleen cells. Whilst in the PLD inoculated goats, the histopathological examination of the spleen showed edema with mild presence of inflammatory cells. In addition, there were few spots of distinct degeneration and necrosis scattered in the red pulp of the spleen. The current findings are by some means with agreement of those reported by Osman *et al*. (2012) that inoculation of the PLD in mice resulted in edematous and hemorrhagic spleen. Based upon histopathological examination, the spleen tissues showed severe congestion, hemorrhage and infiltration of inflammatory cells.

Caseous lymphadenitis is a disease of lymphatic system caused by the *C. pseudotuberculosis* infection and resulting in abscess formation in the lymph nodes (Paton, 2010). Rams naturally infected with the *C. pseudotuberculosis* exhibit classical lesions of CLA such as enlargement of torso lymph nodes with many subcutaneous abscesses spread all over the body. Upon cut, the lymph nodes revealed green yellowish pus and some of the lymph nodes exhibit the characteristic onion rings pattern (Ibtisam, 2008). In our study, the *C. pseudotuberculosis* infection caused abscessation in the external and internal lymph nodes of the goats. The first histopathological changes in the lymph nodes of sheep and goats are after the *C. pseudotuberculosis* infection is infiltration of large number of neutrophil and eosinophil with formation of micro-abscesses (Valli, 1993). Noteworthy that none of the lymph nodes from the *C. pseudotuberculosis* infected goats in this study developed the virtually classic onion rings presentation that is pathognomonic for CLA in sheep. Mice infected with the *C. pseudotuberculosis* also developed macro-abscesses in the abdominal lymph nodes and most of the visceral organs. Our findings agree with those of Osman *et al*. (2012) in mice and Ibtisam (2008) in rams, both reported that the cellular changes were edema, congestion in the cortex and the medulla of the lymph nodes, degeneration and necrosis of the lymphatic tissue along side with the presence of micro-foci of abscess formation and depletion of the lymphoid follicles.

Inoculation of the PLD in mice resulted in bloody diarrhea, serous eye discharge and swelling of the cranio-cervical area. The cellular changes in the abdominal lymph nodes were severe congestion and hemorrhage, edema and infiltration of inflammatory cells. However, there was no formation of any foci of micro abscess in the abdominal lymph nodes (Osman *et al*., 2012). In the current study, goats inoculated with the PLD did not develop any abscesses in their lymph nodes neither in their visceral organs. Histopathological examination showed a mild presence of inflammatory cells and hemosiderin deposits were seen in some of the lymph nodes. Similarly, *Loxosceles recluse*, also known as North American brown recluse spider, its venom has high similarity to *C. pseudotuberculosis* exotoxin, PLD. It causes dermonecrosis, renal failure, platelet aggregation and hemolysis (Lee and Lynch, 2005). We believe that the PLD acts in similar way of brown recluse spider venom resulted in hemolysis and hemosiderin production in the lymphatic tissues.

Conclusion

The current study concluded that the *C. pseudotuberculosis* infection in the goats produces typical caseous lymphadenitis disease with short incubation period of two weeks marked by the presence of abscess formation at the inoculation site and other superficial lymph nodes confined mostly to the head

region. However, the PLD inoculation showed some general clinical signs and the animals did not develop or exhibit any abscesses internally or externally. We believed that the PLD plays a key role in CLA development, yet it is impossible to trigger granulomatous lesion without the *C. pseudotuberculosis* presence. In addition, the findings of the *C. pseudotuberculosis* infection are similar with almost all previous studies. However, the findings of the PLD inoculation in goats were new to report and add merits to the knowledge of CLA disease in goats. Both, the *C. pseudotuberculosis* infection and the PLD challenge in goats caused significant gross manifestations in visceral organs. Moreover, histopathological examination revealed significant and relatively new findings. Furthermore, while the disease has been reported in Malaysia since early 1970s, there is scanty research concerning CLA in sheep and goats. Caseous lymphadenitis in goats is almost similar of that in sheep; however, in the current study, goats did not develop the virtually classic onion rings presentation that is pathognomonic for CLA in sheep. Strikingly, the PLD effects on different body systems, organs and their tissues were consistent and this study is the first to report the cellular changes in goat's visceral organs post inoculation with the PLD. Consequently, we believe that this new findings will provide better understanding of the pathogenesis of CLA in goats and it will enlighten the future research of CLA.

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Author's Contributions

F.F. Jesse: Contributed to the design of the field trial.

Z.K.H. Jeber: Ran the experiment and collect the samples.

Z.K.H. Jeber and F.F. Jesse: Analyzed the results and drafted the paper.

F.F. Jesse, A.A. Saharee, J. Sabri, R. Yusoff and H. Wahid: Have contributed to the design of the study, writing the manuscript and coordination of the study. All authors have read and approved the manuscript.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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