The Pharma Innovation Journal 2022; SP-11(7): 4072-4075

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(7): 4072-4075
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www.thepharmajournal.com
Received: 20-04-2022
Accepted: 24-05-2022

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Pathomorphological studies of caseous lymphadenitis in camel (*Camelus dromedarius*)

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Abstract
The present study was undertaken to determine the occurrence and pathomorphological changes of caseous lymphadenitis in camel in North-Western, Rajasthan. Majority of the camels included in this study had died of natural causes. The overall occurrences of this conditions were 27.58% reported in present study. Grossly, the lymph nodes were congested, swollen and enlarged. It contains odorless, non-granular and non-calcified thin creamy homogenous yellowish white pus in cortex and medulla. Histopathological, homogeneous eosinophilic fluid contains fragmented nuclear, large numbers of neutrophils, lymphocytes and cellular debris with partial and complete depletion of lymphocytes from germinal center. Gram positive 5(27.77 per cent) and Gram negative 13(72.22 per cent) were identified. The most predominant microorganisms were *Klebsiella pneumoniae* 27.77 per cent, followed by *Enterobacter cloacae* 22.22 per cent, *E. coli* 16.66 per cent, *Enterococcus* spp., *Bacillus* spp., 11.11 per cent, *S. aureus* and *Stenotroph maltophillia* 5.55 per cent reported.

Keywords: Lymph nodes, gross, histopathology, camel

1. Introduction
Caseous lymphadenitis is a chronic contagious and non-fatal bacterial infection. It caused abscesses in external and internal lymph nodes. These abscesses enlarge, may ruptured and discharge infectious pus. The disease is caused mainly by a Gram-positive intracellular coccobacilli bacteria called *Corynebacterium pseudotuberculosis* previously known as *Corynebacterium ovis* [Chaudhary 2017] [1]. Camel infections with pyogenic bacteria such as *Corynebacterium pseudotuberculosis*, *C. pyogenes*, *E. coli* group B streptococci and staphylococci have been reported. Abscesses are common in dromedaries, particularly in the form of lymphangitis accompanied by caseous lymphadenitis [Werney, 2012] [2]. The causative organism enters through the damaged skin and mucous membrane and finally reaches the regional lymph nodes and causes inflammatory and necrotic changes. Degenerative and chronic diseases cause internal subclinical lymph nodes lesions which are generally reported during natural death. Infection is usually a wound contamination, but inhalation and ingestion were also reported (Wernery, 2012) [2]. Progressive weight loss, carcass trimmings and skin condemnation at abattoirs are negative economic effects of the disease on production and trading (Borham, *et al*. 2016) [3]. Affected camels may remain subclinical or show apparent symptoms. CLA lesions usually appear as abscesses in the superficial or visceral lymph nodes associated with chronic weight loss (Hawari, 2008) [4]. However. The CLA in small ruminants and camelids is characterized by the formation of onion ring pattern abscesses in one or more superficial lymph nodes and may also extend to internal organs such as the lung and liver. However, moderate to severe abscessation of joints, subcutaneous, tissue and muscles were also reported. The abscesses were encapsulated with a thick capsule, containing odourless, non-granular, noncalcified and thin creamy yellowish-white pus. Histologically, suppuration, necrosis, hyperplastic lymphoid follicles and partial and complete depletion of lymphocytes from germinal centre were observed (Werney, 2012) [2].

2. Materials and Methods
In present study, a total (n=170) samples of lymph node were examined, out of them 30 tissue showing frank gross lesions were collected in 10 per cent formal saline for further gross and histopathological examination. The tissues were processed for paraffin embedding by acetone and benzene technique {Lillie, 1965] [5]. The tissue sections of 4-5 µm thickness were cut by the help of manual microtone and stained with haematoxylin and eosin staining method [Luna, 1968] [6]. The possible results were recorded by both grossly and histopathologically.

2.1 Collection of samples for bacteriological work

Tissue swabs (n=21) from gross lesions for bacteriological work collected. The tissue piece was prepared by removing the surrounding fat and fascia by sterile scissor and forceps. Examined the tissue for gross lesions and made a deep incision by help of sterile surgical blade. Absorbent cotton swab swabbed from deep lesions and kept in sterile tube contained 2-4ml normal saline/nutrient broth. Transport the collected samples in laboratory as soon as possible in ice pack box and incubated overnight at 37 ºC. The plates were examined for the presence of growth after 24-48hr, then smeared for Gram stain to identify Gram-positive and negative bacteria. The isolates were confirmed by using (MALDI-TOF) MS (VITEK MS RUO) system.

3. Results

3.1 Histopathologically

3.1.1 Grossly: The lymph nodes were slightly congested, swollen and marked enlarged without abscesses formation on external surface. On longitudinal incision, it contained odorless, non-granular and non-calcified thin creamy homogenous yellowish white pus in cortex and medulla (fig. 1). Various size abscesses were seen (barely to orange size) on inner surface of lymph nodes involving cortex and medulla (fig.2).

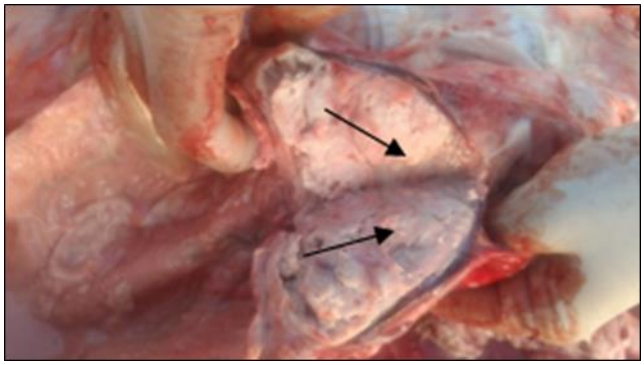

Fig 1: Gross photograph of lymph node showing creamy thin white pus occupied in cortex and medulla

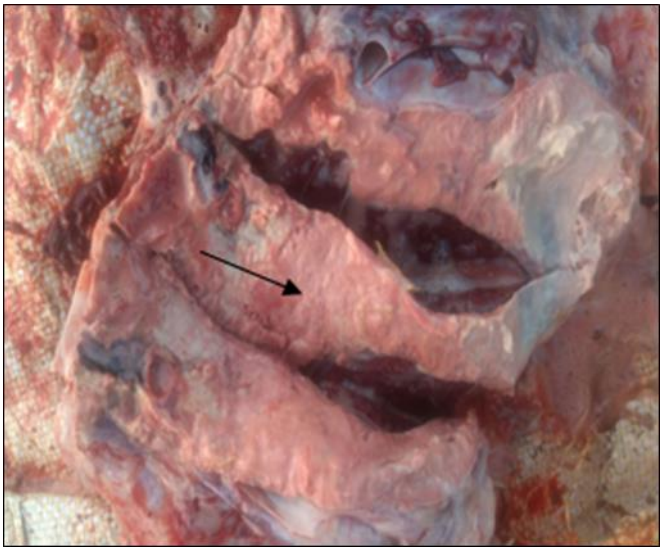

Fig 2: Gross photograph of longitudinal section of lymph node showing multiple size abscess in cortex and medullary region

3.1.2 Microscopically: The germinal follicles contained necrotic center surround by thin zone of macrophages, lymphocytes and encapsulated with fibrous capsule (fig. 3). The pus appeared as homogeneous eosinophilic fluid contains fragmented nuclear, large numbers of neutrophils, lymphocytes and cellular debris (fig. 4, fig 5). It showed partial and complete depletion of lymphocytes from germinal center (fig. 6). Edema of the lymphoid follicles was also reported.

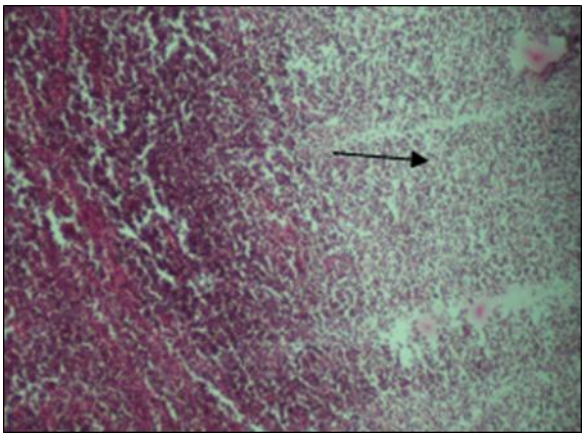

Fig 3: Microphotograph of lymph node showing caseous necrotic areas in germinal centre, surrounded by macrophages (10x)

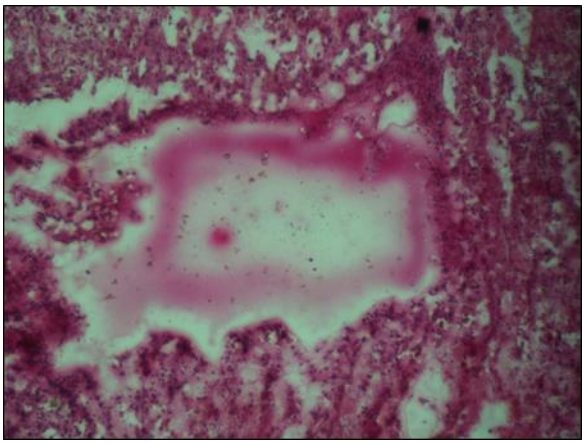

Fig 4: Microphotograph of lymph node showing homogeneous eosinophilic fluid contains fragmented nuclear, large numbers of neutrophils, lymphocytes and cellular debris (10x)

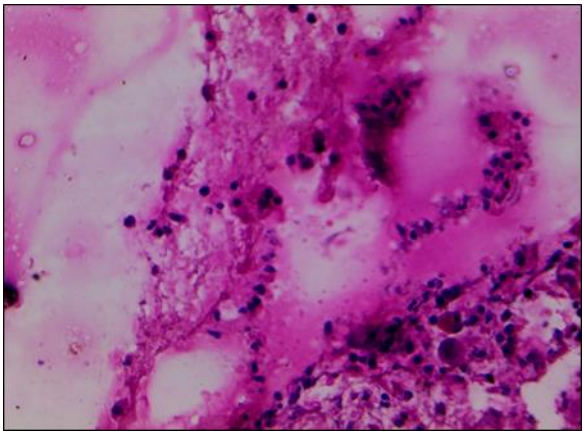

Fig 5: Higher magnification of fig. 5 (400x) showing cellular debris and infiltration of macrophages and neutrophils

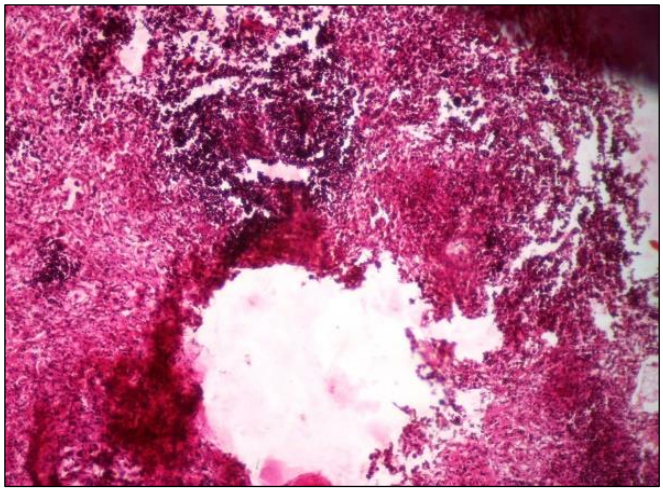

Fig 6: Microphotograph of lymph node showing complete depletion of lymphocytes from germinal center.10x

3.2 Bacteriologically

Twenty-one tissue swabs (n=21) were taken from the abnormal lymph nodes in camel carcasses. The (n=15, 71.42%) samples were bacteriological culture positive when inoculated onto nutrient agar plates, aerobically for 24-48 hours at 37 °C. Moreover (n=6, 28.57%) samples didn't show any bacterial growth up to 48 hours after incubation. A total number of 18 isolates were isolated. It included Gram positive cocci, bacilli and Gram-negative bacilli, belong to 7 genera were reported in this study. Bacterial spectrums of 3(20%) positive culture samples were not identified by MALDI-TOP MS, due to mixed culture or contaminations.

Table 5: The percentage of microorganisms isolated from caseous lymphadenitis in camel

Isolated organisms	No. of isolates (n=21)	Percentages
E. coli	3	16.66
K. pneumoniae	5	27.77
Bacillus spp.	2	11.11
Enterobacter cloacae	4	22.22
Stenotroph maltophillia	1	5.55
Enterococcus spp.	2	11.11
S. aureus	1	5.55
Total	18	100

Out of total 18 isolates, Gram positive 5(27.77 per cent) and Gram negative 13(72.22 per cent) were identified. More than one isolates were reported from some samples. A total of 18 bacterial isolates were obtained from (n=21) samples.

Among Gram positive bacteria higher incidence of *Bacillus* spp., *Enterococcus* spp., 40 per cent each followed by *S. aureus* 20 per cent were reported from total Gram-positive bacteria. Among Gram negative bacteria higher incidence of *K. pneumoniae* 38.46 per cent followed by *Enterobacter cloacae* 30.76 per cent, *E. coli* 23.07 per cent and *Stenotroph maltophillia* 7.69 per cent were reported from total Gram-negative bacteria.

4. Discussion

The prevalence of caseous lymphadenitis was 17.64% was reported in present study, lower values were reported previously as 10.9% in Egypt (Abou-Zaid *et al*., 1994) [7], 10% in Ethiopia (Domenech *et al*., 1977) [8] and 12% in Sudan (Aljameel *et al*., 2013) [9]. The prevalence of the disease is higher than previous report. It affected by many factors which influence its endemicity including: management procedures, hygienic measures, ecology and awareness about the importance. In Rajasthan camels generally reared on loose pasture grazing in arid and semiarid areas and used for baggage carrying. The contamination of superficial skin wounds caused by shearing cuts, saddle and baggage and thorny injury. So pathogenic bacteria invade the tissue and reach in blood circulation and settle down in lymph nodes.

The superficial form was significantly more prevalent than the visceral form which is attributed to the superficial injuries as the main predisposing factor for CLA. Consequently, superficial form is more prevalent (Al-Gaabary *et al*., 2010) [10]. Similar findings were recorded (Wernery, 2012) [2] who found that CLA lesions are rare in internal organs of camels. Ticks and mange infestation was reported in most of the examined camels. It may predispose for wound-infection by tick-bite injury as well as mange-induced injuries. Camels may rub their heads and necks to the wire fences which predispose for abscess formation. Similarly, many authors had reported the same findings (Radwan *et al*., 1989; Zidan *et al*., 2013) [11, 12].

Variation in the lesion size is dependent on many factors. The stage of the disease, potency and amount of the bacterial exotoxin in addition to the immune status of the animal. Size variation of CLA lesions is a common feature of the disease (Wernery, 2012; Dioli, 2013) [2, 13]. Hardness and painless nature of the majority of CLA lesions is attributed to the chronic nature of the disease as old abscesses become more consistent with tendency toward fibrosis and calcification.

Maddy (1953) reported that internal lymph node abscess in camels might be attributed to drinking of contaminated water from stagnant pool. The distribution of the visceral lesions revealed the affection of lung, bronchial and mediastinal lymph nodes. These findings were described in sheep (Binns *et al*., 2007; Fontaine and Baird, 2008) [14, 15]. Who reported that mediastinal lymph node and lungs are the most common visceral lesion sites. Consequently, inhalation is the probable route of infection in such cases. Congestion without abscess formation was recorded in previous studies (Hawari, 2008, Radwan *et al*., 1989) [4, 11]. The creamy whitish color of the pus was referred to the highly liquefactive phagocytic enzymes of camels (Radwan *et al*., 1989; Dioli, 2013) [11, 13].

The replacement of lymphoid follicles, liquefactive necrosis and hyperemia of lymphoid tissue, edema of lymph channels and hyperplasia of lymphoid cords and their dilation, caseous central necrosis and pyogranulomatous lesions with aggregation of macrophage within the sinusoid reflect the nature of the disease as a chronic suppurative condition. Similarly, suppurative lymphadenitis and liquefactive necrosis in addition to the aggregation of a large number of macrophages and neutrophils were previously reported (Zidan *et al*., 2013) [12].

Isolation of organisms is mainly due to the chronic nature of lesions as old abscesses usually contain low numbers of viable organisms and become nearly sterile (Baired and Fontaine, 2007; Oreiby *et al*., 2013) [16, 17]. It indicates either its role as a primary or secondary pathogen.

In conclusion, the nature of CLA lesions in camels is variable and may appear as congestion, abscessation or scar formation.

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