



Caseous Lymphadenitis in Sudanese and Somalian Camels Imported for Meat Consumption in Egypt

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ABSTRACT

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This study was conducted on Sudanese and Somalian imported camels at Al-Warraaq abattoir, Giza (Egypt), during the period from September 2014 to November 2015. A total of 850 adult one-humped camels (*Camelus dromedarius*) were subjected to clinical and postmortem examinations. Caseous lymphadenitis lesions were detected in 88 (10.35%) animals. Superficial form (9.76%) was significantly more prevalent than the visceral form (0.58%). Incised lesions showed cheesy greenish-whitish pus, four lesions were congested without abscess formation. Coryneform bacteria were isolated as a pure culture in 11 (30.55%) cases. Targeting *Corynebacterium pseudotuberculosis* serotype 1, being a common cause of caseous lymphadenitis, two isolates were identified by *pld* gene-specific polymerase chain reaction. Other coryneforms were tested by the API Coryne system; three *Cellulomonas* spp./*Microbacterium* spp. and one *Brevibacterium* spp. were identified. The API-identified bacteria were subjected to the Vitek 2 system which identified two isolates as *Corynebacterium jeikeium* and *Corynebacterium urealyticum*. The Vitek 2 system-identified isolates were classified as unidentified coryneforms. Histopathological findings revealed suppuration, necrosis, hyperplastic lymphoid follicles and sinus histiocytosis of the affected lymph nodes. In contrast to small ruminants, few studies are available on caseous lymphadenitis in camel and rare of them involve the visceral form. In conclusion, caseous lymphadenitis is prevalent in Sudanese and Somali imported camels in Egypt. In camels, the disease has variable natures and visceral lesions are uncommon. Coryneform bacteria other than *Corynebacterium pseudotuberculosis* are associated with the disease in camels which necessitate multiple identification steps.

1. INTRODUCTION:

Caseous lymphadenitis (CLA) is a chronic bacterial disease of camelids, wild and domestic small ruminants, pigs and man (Baird and Fontaine, 2007; Colom-Cadena, et al. 2014; Oliveira, et al. 2014). In camelids, it had been reported in old world camels: *Camelus dromedarius* and *Camelus bactrianus* as

well as in new world camels: Llamas and alpacas (Wernery, 2012). *Corynebacterium* (*C.*) *pseudotuberculosis* serotype 1 is the main causative bacterium of CLA in different animals. However, other pathogens such as *C. pseudotuberculosis* serotype 2, *C. ulcerans*, many other corynebacteria, *Streptococcus* Spp. and *Staphylococcus* spp. may be involved in CLA of camels (Tejedor et al., 2000;

Tejedor-Junco et al., 2008; Aljameel et al., 2013; Zidan et al., 2013). Infection is usually a wound contamination, but inhalation and ingestion were also reported (Wernery, 2012). Progressive weight loss, carcass trimmings and skin condemnation at abattoirs are negative economic effects of the disease on camel production and trading (Borham, et al. 2016). Affected camels may remain subclinical or show apparent symptoms. CLA lesions usually appear as abscesses in the superficial or visceral lymph nodes and organs associated with chronic weight loss (Hawari, 2008). However, visceral involvement and the onion ring pattern of CLA abscess, which is common in sheep and goat, has been rarely described in camels (Abou-Zaid, et al. 1994; Wernery, 2012; Aljameel, et al. 2014). Confirmatory diagnosis of the disease depends on bacteriological culture and identification of the causative bacteria. In contrast to small ruminants, studies involving CLA in camels are scarce. In Egypt, limited studies had been conducted on the disease in native camels and to our knowledge, there are none involved the imported camels. So, this study was conducted to detect the prevalence, clinical and postmortem findings associated with CLA in Sudanese and Somalian camels imported for meat consumption in Egypt. In addition, to describe the disease associated tissue changes and to identify the causative coryneform bacteria.

2. MATERIALS AND METHODS:

2.1. Animals

A total of 850 adult one-humped camels (*Camelus dromedarius*), imported from Sudan and Somalia for meat consumption and slaughtered at Al-Warraaq abattoir, Giza (Egypt), were subjected to clinical and postmortem examinations (Hawari, 2008; Dioli, 2013).

2.2. Sampling and bacteriological isolation

A total of 96 suspected lesions; 91 superficial lesions and 5 visceral lesions: 1 bronchial lymph node, 1 mediastinal lymph node and 3 lung abscesses were aseptically collected from 88 animals. The lesions were transported to the lab on ice. A loopful of pus from each lesion was streaked over brain heart infusion agar and incubated aerobically at 37°C for 48-72 h (Tejedor et al., 2004). Gram-stained bacterial smears were prepared from the resultant colonies and Gram-positive non-spore forming bacilli and

coccobacilli bacteria were kept for identification; firstly, a specific polymerase chain reaction targeting *C. pseudotuberculosis*, followed by the biochemical identification of PCR- unidentified coryneform bacteria.

2.3. Polymerase chain reaction (PCR)

Extraction of deoxyribonucleic acid (DNA) was performed by QIAamp® DNA Mini Kit (Qiagen, Germany) on 11 pure coryneform bacteria isolates. Concentration and purity of the extracted DNA samples were determined by measuring absorbance at 260 and 280 nm using a BIO-RAD biophotometer (Biorad, USA). A highly specific PCR targeting *pld* gene (203 bp) of *C. pseudotuberculosis* was performed (Ilhan et al., 2013). A reaction volume of 25 µl containing 6 µl template DNA, 12.5 µl EmeraldAmp® GT PCR master mix (Takara, Japan) and 20 pmol of *pld*-F (5'-ATA AGC GTA AGC AGG GAG CA-3') as well as *pld*-R2 (5'-ATC AGC GGT GAT TGT CTT CCA GG-3'). Cycling was performed under the following conditions; initial heating at 94°C/5 min followed by 35 cycles of 94°C/30 s, 56°C/30 s and 72°C/30 s. The final extension was performed at 72°C for 5 min. PCR negative coryneform bacteria were subjected to biochemical identification.

2.4. Biochemical identification

The API® Coryne system kit (bioMérieux, Marcy-L'Etoile, France) were used for the identification of the isolated Gram-positive non-spore forming rods which were not identified by PCR targeting *pld* of *C. pseudotuberculosis*. Some isolates couldn't be identified by the API® Coryne system and were subjected to the Vitek 2 system that uses fluorescence based technology according to (Biomerieux user guide 2006, France). However, some isolates couldn't be identified by any of the previous methods and were classified as unidentified coryneforms.

2.5. Histopathological examination

Ten tissue samples (5 affected lymph nodes, 4 skin samples and one lung lesion) were fixed in 10% buffered formalin solution, dehydrated in graded ethanol and embedded in paraffin wax. Five microns thick sections were prepared and stained by hematoxylin and eosin for the microscopical examination (Hewitson and Darby, 2010).

2.6. Statistical analysis

Chi-square was performed using SPSS 21.

3. RESULTS:

3.1. Disease frequency

Out of the 850 examined slaughtered animals, 88 (10.35%) camels were found affected. Both Superficial and visceral forms had been detected. However, the superficial form was significantly ($p < 0.00001$) more prevalent than the visceral form where they were recorded in 83 (9.76%) and 5 (0.58%) animals, respectively.

3.2. Clinical nature of CLA in camels

It was observed that most of the examined camels were suffering from heavy tick infestation and some of them had mange. The affected animals showed enlargement of different superficial lymph nodes with a distinct variation in size ranging from small lemon up to orange size or even larger. Out of the 83 superficial lesions affected camels, 73, 8 and 2 animals had a single, double and multiple superficial lesion in a percentage of 87.95%, 9.63% and 2.40%, respectively. Those cases of multiple superficial lesions showed ulceration and large scar formation, particularly at the base of the neck. The distribution of the detected lesions is shown in Table 1.

Out of the detected lesions, 90 (93.75%) were closed. However, 6 (6.25%) lesions were opened

discharging pus, associated with the ulceration of the overlying skin. The opened lesions were of large size (up to orange size) and were doughy in texture. The pus was of whitish creamy or greenish colour and milk-like, thick-cheesy or dry and firm in consistency. All lesions were cold and painless except for 4 lesions which were hot and painful. Clinical signs of CLA in camels are shown in Fig. 1-4.

3.3. Postmortem examination findings

Out of the 850 slaughtered camels, 5(0.58%) animals had visceral lesions and none of them had showed superficial abscesses or clinical evidence of visceral involvement. Lung, bronchial and mediastinal lymph nodes were the affected visceral sites. Multiple pulmonary abscesses were detected in two cases. Two cases had mediastinal lymph node lesions, in another case, lung and bronchial lymph node lesions were observed.

Upon incision of superficial and visceral lesions, cheesy or dry-firm pus of greenish or whitish colour was evident. Onion ring arrangement of the lesions could not be detected. Three superficial and one mediastinal lesions were enlarged and congested without abscess formation. Postmortem findings of CLA in camels are shown in Fig. 5-8.

Table 1. Distribution of the superficial caseous lymphadenitis lesions.

LN*	No. of lesions	%
Inferior cervical	78	81.25%
Prescapular	10	10.41%
Tuberal	3	3.12%
Popliteal	2	2.08%
Mandibular	2	2.08%
Pectoral	1	1.04%

*Lymph node

Table 2. Different isolates based on its morphology as revealed by Gram stain.

Isolate	No. n=36	% of isolates	% of Cases n=88
Gram-positive non-spore forming rods	11	30.55%	12.5%
Mixed Gram-positive rods and cocci	4	11.11%	4.54%
Gram-positive cocci	21	58.33%	23.86%



Fig. 1. Unilateral inferior cervical lymph node lesion.



Fig. 2. Right inferior cervical and prescapular lymph nodes lesions associated with ulceration of the overlying skin.



Fig. 3. Bilateral inferior cervical lymph nodes lesions in association with bilateral prescapular lymph nodes lesions and skin ulceration.



Fig. 4. Bilateral tubercular lymph nodes lesions.

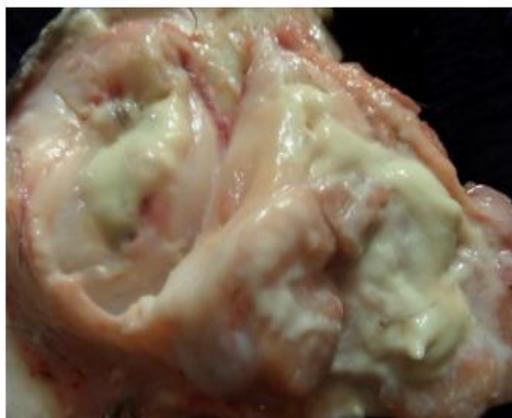


Fig. 5. Fig. 5. Incised mandibular lymph node lesion showing greenish cheesy pus.



Fig. 6. Incised inferior cervical lymph node lesion showing enlargement and congestion without abscess formation.



Fig. 7. Incised mediastinal lymph node lesion showing enlargement and congestion without abscess formation.



Fig. 8. Pulmonary lesion showing multiple small caseous abscesses.

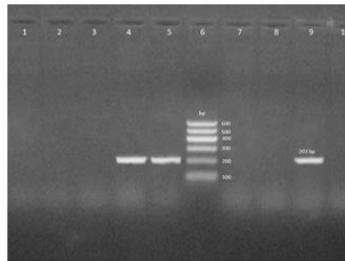


Fig. (9): PCR targeting 203 bp amplicon of *C. pseudotuberculosis* PLD gene; lane 5: control positive, lane 10: control negative, lane 4 and 9: positive isolates and lane 1, 2, 3, 7 and 8: negative isolates.

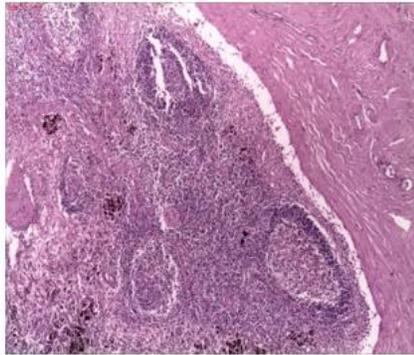


Fig. (10): Area underneath intact skin showing reactive lymph node with hyperplastic lymphoid follicles and sinus histiocytosis (H&E x40).

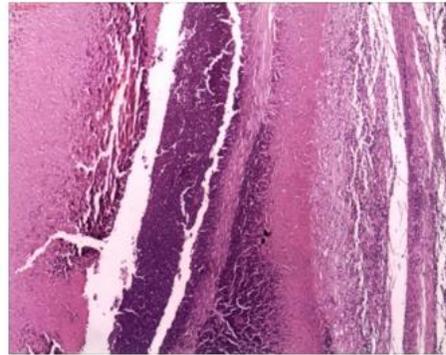


Fig. (11): Section in lymph node showing marked suppuration and necrosis. (H&E x40).

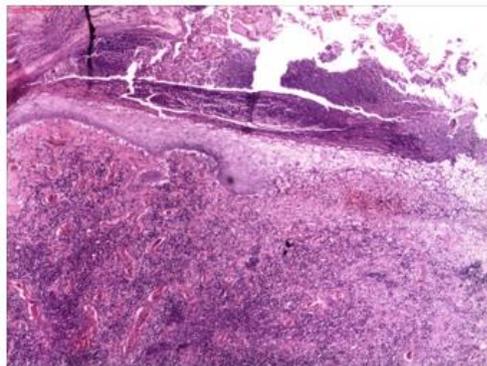


Fig. (12): A figure showing acute suppurative inflammation of the skin with area of ulceration, (H&E x40)

3.4. Identification of the isolated coryneform bacteria

Out of the 88 affected camels, bacteria had been isolated from 36 cases (40.9%). On the other hand, 52 (59.1%) cases did not yield any isolates. Isolates based on its morphology and its percentages of the total isolates and the affected cases are shown in Table 2.

All Gram-positive bacilli isolates had similar culture characters: mucoid, opaque, greyish-white colonies. The bacilli arranged as Chinese letters or V-shape. Identification of the 11 pure-culture isolates had passed through three successive identification steps. Firstly, targeting *pld* gene by PCR, two isolates were identified as *C. pseudotuberculosis* serotype 1 (Fig 9.). Other isolates were tested by the API Coryne system, four of them were identified: three *Cellulomonas* spp. /*Microbacterium* spp. isolates (API profiles 7652775, 3540775 and 7052337) and one *Brevibacterium* spp. (API profile 4102004). The five API-unidentified bacteria were subjected to the Vitek 2 system, two isolates were identified as *C. jeikeium* and *C. urealyticum* with a very good and excellent identification confidence, respectively. Three isolates were unidentifiable by both the API Coryne system and the Vitek 2 system and were classified as unidentified coryneforms.

3.5. Histopathological findings

Histopathological examination showed marked suppuration, necrosis, hyperplastic lymphoid follicles and sinus histiocytosis of the affected lymph nodes. In addition, skin lesions showed suppurative inflammation and ulceration. The affected lung tissue showed suppurative focus with central necrosis and areas of acute inflammatory infiltrate around bronchioles beside congested vessels. Some histopathological findings are shown in Fig. 10-12.

4. DISCUSSION

CLA appears to be of nearly similar endemicity in the eastern African countries. A prevalence of 10.35% was reported, similar values were reported previously; 10.9 % in Egypt (Abou-Zaid et al., 1994), 10% in Ethiopia (Domenech et al., 1977) and 12 % in Sudan (Aljameel et al., 2013). The prevalence of the disease is affected by many factors which influence its endemicity including: management procedures, hygienic measures, ecology and awareness about the importance and the ideal

procedures taken to limit the disease spread.

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 and in a lower number of cases hematogenous or lymphogenous spread may occur (Al-Gaabary et al., 2010). Similar findings were recorded (Wernery, 2012) who found that CLA lesions are rare in internal organs of camels.

Tick infestation was reported in most of the examined camels and some of CLA-affected camels had suffered from mange. This may predispose for wound-infection of tick-bite injury as well as mange-induced injuries resulting from repeated rubbing against hard objects. Similarly, many authors had reported the same findings (Radwan et al., 1989; Guimarães et al., 2011; Zidan et al., 2013).

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 (Schwartz et al., 1982; wernery, 2012; Dioli, 2013). 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. However, fewer numbers of lesions were hot and painful. Similar finding was reported in camels (Moussiaux et al., 2005; Moallin, 2009). The pain and hotness of these lesions are because of inflammatory mediators and may be indicative of the early stages of the disease. Moreover, most of the detected lesions were closed which is mainly because of the slow ripening nature of the CLA lesion in addition to the thick camel's skin.

Considering the number of superficial lesions per animal as well as the absence of concurrent superficial and visceral lesions, it is obvious that metastasis is not a common feature of the disease. CLA-causative pathogens are trapped at the primary site of infection, in a few cases metastasis to other sites may occur (Zidan et al., 2013). Additionally, the possibility of concurrent infection at two or three different sites may occasionally exist (Oreiby, 2013). The distribution of lesions indicates that the anterior body half affection is common, particularly in the cervical region (Inferior cervical and prescapular lymph nodes). Similar results were reported in previous studies (Hawari, 2008; Moallin, 2009). The

cervical region is highly exposed to abrasions which may occur because of friction against hard and sharp objects during the transportation of camels in merchant ships or from seaports to markets and abattoirs as camels are transmitted in the sternal recumbency position.

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) 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. Some affected lymph nodes showed congestion and enlargement without abscess formation which may indicate a different nature or the early stage of the disease. Congestion without abscess formation was recorded in previous studies (Radwan et al., 1989; Hawari, 2008).

The creamy whitish color of the pus was referred to the highly liquefactive phagocytic enzymes of camels (Radwan et al., 1989; Dioli, 2013). In addition, greenish color of pus was also reported, in contrast to what was stated in a previous study (Hawari, 2008) who mentioned that the greenish color is characteristic for the disease in sheep not in camels. In sheep, the greenish color was attributed to a greenish pigment produced by *C. pseudotuberculosis* or due to the existence of huge numbers of eosinophiles (Fontaine and Baird, 2008).

Isolation failure in 59.1% of cases is mainly due to the chronic nature of lesions as old abscesses usually contain low numbers of viable organisms and become nearly sterile (Baird and Fontaine, 2007; Oreiby et al., 2013). Isolation of Gram-positive cocci as a sole isolate from 23.86% of cases indicate either its role as a primary or secondary pathogen as its presence overshadow the sensitive coryneform bacteria (Mubarak et al., 1999). Also, this explains the lower isolation rate of *C. pseudotuberculosis* where two isolates were identified by PCR as *C. pseudotuberculosis* out of 11. Similar results were recorded by Zidan et al. (2013) who had isolated two *C. pseudotuberculosis* out of 16 *Corynebacterium* spp. isolates from camels. The identification of many coryneform bacteria other than *C. pseudotuberculosis* point-out to its role as causative pathogens in camels (Zidan et al., 2013). Similarly, in sheep *C. urealyticum* was recently isolated from a CLA lesion (Huerta et al., 2013). However, it is well known that

C. pseudotuberculosis is the main cause of the disease in sheep, but this is not the situation in camels.

The hyperplasia of lymphoid follicles, necrosis and the aggregation of macrophage within the sinusoid of lymph node 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).

In conclusion, CLA is prevalent among Sudanese and Somalian imported camels for meat consumption in Egypt. The nature of CLA lesions in camels is variable and may appear as congestion, abscessation or scar formation. In addition, visceral CLA lesions are uncommon in camels. Many coryneform bacteria other than *C. pseudotuberculosis* are associated with CLA in camels which necessitate multiple identification steps.

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