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PREVALENCE AND ANTIBIOTIC SENSITIVITY OF CORYNBACTERIUM PSEUDODTUBERCULOSIS ISOLATED FROM CAMELS SLAUGHTERED IN THREE MAJOR ABATTOIRS AT CAIRO AND GIZA

By

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ABSTRACT

Corynebacterium pseudotuberculosis (*C. pseudotuberculosis*) is the causative agent of caseous lymphadenitis, a chronic suppurative disease with a worldwide distribution, in sheep, goats and camels. This study was conducted to investigate the prevalence of *C. pseudotuberculosis* in affected lymph nodes of camels slaughtered at El-Basatin, El-Waraq and Kerdasa abattoirs in Cairo and Giza Governorates, Egypt. Meanwhile, the antimicrobial resistance profiles of the bacterial isolates were investigated for better control of the condition in live animals. Out of 792 camel carcasses examined, 92 were affected with caseous lymphadenitis. The visceral form of the disease was detected in 69 carcasses (75%) while the peripheral form was found in 23 carcasses (25%). Concerning age categories, the affection was more prevalent in camels less than seven years old. Based on the bacteriological investigation, the prevalence rates of *C. pseudotuberculosis* among the affected carcasses of was 18.48% (17 carcasses). Results of the antibiogram showed that all isolates were sensitive for norfloxacin (100%) and moderatly sensitive for piperaciliine (54.55%). High level of resistance was recorded against penicillin G (100%), followed by emoxiclave (90.90%) and gentamycin, rifampicin and vancomycin (81.82% for each). Some isolates inferred resistance against more than one antibacterial agents indicating the alarming existence of multiple drug resistance of *C. pseudotuberculosis*.

Key words:

Camels, caseous lymphadenitis, *C. pseudotuberculosis.*

INTRODUCTION

Caseous lymphadenitis (CLA) is a chronic disease caused by *C. pseudotuberculosis*. The pathogen has a broad spectrum of hosts and causes economic losses in sheep, goats, cattle, horses and camels **(Moore *et al.*, 2010)** due to abscesses formation in one or more superficial or internal lymph nodes and internal organs **(Al-Jameel *et al.*, 2013).** Abscesses in the internal organs are only detected after the animal slaughter as even hundreds of small abscesses or several large abscesses rarely cause clinical manifestation **(Nasgaraja and Chengappa, 1998).** The World Animal Health Organization (OIE) declared that 64 countries had animals with caseous lymphadenitis within their borders. These countries belong to Americas (19 of 42 countries), Oceania (2 of 14), Asia (11 of 43), Europe (14 of 51) and Africa (18 of 51) **(OIE, 2009).** Camels represent a major source of meat in many countries all over the world. More than 80% of camel population inhabits Africa with 60 % in the eastern African countries that include Sudan, Somalia, Kenya, Ethiopia and Egypt **(Faye, 2015).**

Egypt meets much of the demand for camel meat either by local production or mostly from importation **(Kadim *et al.*, 2012).** Dissemination of CLA throughout the world probably occurred through importation of infected animals **(Fontaine (2007).**

There are reports that CLA is endemic in the Middle East and much of Europe **(Brown and Olander, 1987). Afzal *et al.* (1996)** reported that *C. pseudotuberculosis* affects almost 10% of the population in a herd. Endemically, the prevalence of CLA in camels appears to be nearly similar in different countries **(Borham *et al.*, 2017).**

C. pseudotuberculosis is classified into two biovars, the biovar Ovis that is nitrate reduction negative and referred as biotype and the biovar Equi, which is nitrate reduction positive and referred as biotype II **(Biberstein *et al.*, 1971; Barakat *et al.*, 1984; Baird and Fontaine, 2007).** The biovar ovis mainly affects sheep and goats. The most prevalent biovar in camelids has long been described as biovar ovis **(Tejedor *et al.*, 2004)**, but further studies have indicated the susceptibility of dromedary camels to biovar equi **(Tejedor-Junco *et al.*, 2008).**

Virulence factors play an important role in the adhesion, invasion, colonization, spread inside the host, and immune system evasion of pathogenic bacteria **(Schumann, 2007)**. Four *C. pseudotuberculosis* genetic factors have been reported, the *fag*ABC operon and the *fag*D gene. Both enable the bacterium to survive in environments where iron is scarce and found in a pathogenicity island along with the *pld* gene that encodes phospholipase D (PLD) **(Billington *et al.*, 2002; Ruiz *et al.*, 2011).** PLD, a primary virulence factor of

C. pseudotuberculosis, promotes the hydrolysis and degradation of sphingomyelin in endothelial cell membranes increasing the vascular permeability and contributes to the spread and persistence of the bacterium in the host **(Williamson, 2001; Alves and Olander, 1999; Songer *et al.*, 1990).**

The aim of this study was to isolate and identify C. *pseudotubrculosis* from superficial lymph nodes and internal organs of slaughtered camels to investigate the prevalence of caseous lymphadenitis. The susceptibility of the isolates to antimicrobial agents and detection of pld gene were investigated for diagnostic, prophylactic and control purposes.

MATERIAL AND METHODS

Study cases and sampling:

A total of 792 camels slaughtered at El-Basatin, El-Warraq and Kerdasa abattoirs were examined for the presence of abscess lesions in the lymph nodes or internal organs. Suspected lesions were aseptically collected from affected 92 carcasses. Specimens were transferred while cold to the Bacteriology Research Laboratory, Department of Microbiology, Faculty of Veterinary Medicine, and Cairo University with minimum delay.

Isolation and identification of *C. pseudotuberculosis:*

Surface of the lesions was disinfected with 70% ethanol and left for dryness. An incision was made with a sterile scalpel blade and a pus swab was taken from the abscess periphery and streaked onto brain heart infusion agar supplemented with 200 mg/ml fosfomycin and 4 mg/ml nalidixic acid followed by aerobic incubation at 37°C for 48-72 hours **(Zhao *et al.*, 1991).** The suspected bacterial colonies were characterized morphologically and Gram-stained smears were microscopically examined**.** Gram-positive non-spore forming bacilli and coccobacilli isolates were subjected for biochemical identification **(Carter, 1984; Barrow and Feltham; Quinn *et al.*, 2002).** Catalase, urea hydrolysis, nitrate reduction and trehalose fermentation tests were the employed biochemical tests **(Quinn *et al.*, 1994; Koneman *et al.*, 1997).**

Antimicrobial susceptibility testing of *C. Pseudotuberculosis* isolates:

Antimicrobial sensitivity patterns of *C. Pseudotuberculosis* isolates were determined using the Kirby-Bauer disk diffusion method **(Quinn *et al.*, 1994).** The isolates were tested against the commonly available antibiotics. The antimicrobials used and the breakpoint concentrations per disc were: amikacin (30 µg), emoxclave (30 µg), cefotaxime (30 µg), gentamycin (10 µg),

imipenem (10 µg), norfloxacin (10 µg), pencillin G (10 units), piperacillin (100 µg), rifampicin (5 µg) and vancomycin (30 µg).

DNA extraction and PCR assays on C. *pseudotuberculosis* isolates:

DNA was extracted from the bacterial cells using the Gene jet genomic DNA purification kit (Thermo Fisher Scientific Corp., UK) as described by the kit supplier. One milliliter of brain heart infusion broth bacterial culture was transferred to 1.5 ml microfuge tube and centrifuged for 1 minute at 6000 xg. The bacterial pellet was resuspended in 100 µl of 1x PCR reaction buffer followed by heating at 95ºC for 20 minutes and centrifugation for 5 minutes at maximum speed.Two oligonucleotide primers, specific for *C. pseudotuberculosis pld* gene (PLD-F 5'-ATG AGG GAG AAA GTT TTA-3' and PLD-R 5'-TCA CCA CGG GTT ATC CGC-3'), were utilized. PCR reaction was done using Dream Taq polymerase enzyme and through 35 cycles after initial denaturation for 5 min at 95°C. Each cycle consisted of denaturation at 95°C for 30 sec, annealing at 53°C for 30 sec, extension at 72°C for 1 min, and the final extension was for 30 min at 72°C **(Sá *et al*, 2013; Nassar *et al*, 2016; Guerrero *et al*, 2018; Li *et al*, 2018; Cho, 2021).**

The PCR products were electrophoresed in 1% agarose gel containing ethidium bromide (0.5 µg/ml) in TBE buffer. The gel was visualized on a UV transilluminator and photographed by Polaroid MP-4 land camera (Polaroid Corporation, USA).

RESULTS

Incidence of abcsessation and *C. pseudotuberculosis* in slaughtered camels:

Out of 792 examined camel carcasses, 92 (11.61%) showed CLA lesions. Concerning age category, 60 camels (7.57%) were less than 7 years old and 32 (4.04%) were more than seven years old. Bacteriological investigations resulted in the recovery of *C. pseudotuberculosis* from samples of 17 carcasses (1 < 7 years old and 16 > 7 years old) with an overall incidence of 18.47% (Tables 1, 2).

The number of carcasses affected with the visceral form was higher than lesions in the peripheral lymph nodes (69 and 23) representing 75% and 25%, respectively. The inferior cervical lymph node showed the highest incidence (17, 18.47%) and *C. pseudotuberculosis* was isolated from only one sample (1.09%). Lesions were found in 4, 1 and 1 prescapular, popliteal and mandibular lymph nodes (4. 35%, 1.09%, 1.09%), respectively but all resulted in negative isolation of C. *pseudotuberculosis* (Table 3).

C. *pseudotuberculosis* isolates identified on cultural and biochemical characteristics resulted in positive amplification with PCR using *pld* gene specific primers. PCR bands were obtained with the expected 924 bp size Fig. (1).

Table (1): Age distribution of caseous lymphadenitis-like lesions in camel carcasses.

Number of carcasses	Age		Total
	<7 years	>7 years	
Number and ratio of carcasses with internal lesions	52 (6.56%)	17 (2.15%)	69 (8.71%)
Number and ratio of carcasses with superficial lymph node lesions	8 (1.01%)	15 (1.89%)	23 (2.9%)
Total	60 (7.57%)	32 (4.04)	92 (11.61)
Total number of inspected carcasses	638 (80.55%)	154 (19.44%)	792 (100%)

Table (2): Incidence of bacteria in CLA lesions in carcasses of camels of different ages**.**

Number of isolates	Age groups		Total
	<7 years	>7 years	
Number and ratio of bacterial positive CLA lesions in internal lesions	1 (1.09%)	15 (16.3)	16 (17.39)
Number and ratio of bacterial positive CLA lesions in superficial lymph node lesions	0 (0%)	1 (1.09%)	1 (1.09%)
Total number of isolates	1 (1.09%)	16 (17.39%)	17 (18.48%)
Total number of inspected carcasses	60 (65%)	32 (34.78%)	92 (100%)

Table (3): Incidence of C. *pseudotuberculosis* in CLA lesions in internal organs and superficial lymph nodes in slaughtered camels.

Location of the lesions	Internal organs				Superficial lymph nodes lesions					Total
	Lung	Liver	Heart	Total	Inferior cervical	Prescapular	Popliteal	Mandibular	Total	
Number and percentage of CLA lesions	64 (69.56%)	4 (4.35%)	1 (1.09%)	69 (75%)	17 (18.47)	4 (4.35%)	1 (1.09%)	1 (1.09%)	20 (25%)	92 (100%)
***C. pseudotuberculosis* positive lesions**	16 (17.39%)	00	00	16 (17.39%)	1 (1.09 %)	00	00	00	1 (1.09 %)	17 (18.48%)

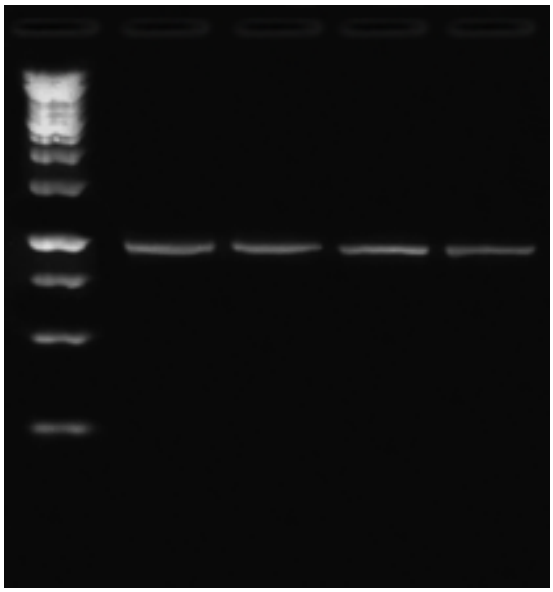


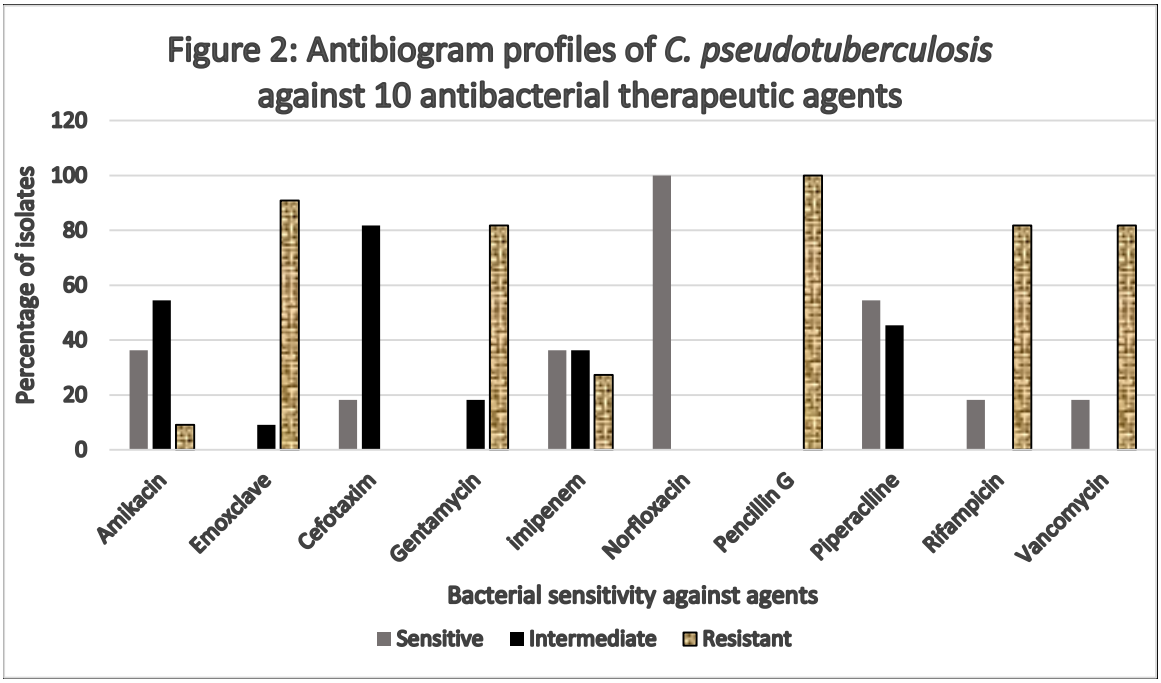
Fig. (1): Agarose gel electrophoresis showing the amplified PCR product for *pld* gene of C. *pseudotuberculosis*. From the left, lane 1: 1Kb DNA size ladder, and lanes 2-5 represent the PCR product of *pld* gene with the expected size (924bp).

Antibiogram of C. *pseudotuberculosis* isolates:

According to the CLSI standards (2015), *C. pseudotuberculosis* isolates showed different antibiogram profiles (Table 4), Fig. (2). Generally, all the examined isolates were highly sensitive to norfloxacin (100%) and moderately sensitive to piperaciliine (54.55%). Amikacin and imipenem were weakly effective (36.36% for each) and the sensitivity percentage decreased to become 18.18 % (Very low effect) with cefotaxim, rifampicin and vancomycin. The majority of the isolates were moderately sensitive to cefotaxim (81.82%). The lowest sensitivity was expressed against gentamycin. Most isolates showed resistance to more than one antibiotic. High level of resistance was recorded against Penicillin G (100%), followed by emoxiclave 10 (90.90%), gentamycin (81.82%) and vancomycin (81.82%).

Table (4): Antibiogram of C. *pseudotuberculosis* isolates from camel CLA lesions**.**

Antibacterial	Degree of sensitivity and isolates numbers and percentages					
	Sensitive		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Amikacin	4	36.36	6	54.55	1	9.09
Emoxiclave			1	9.09	10	90.90
Cefotaxim	2	18.18	9	81.82		
Gentamycin			2	18.18	9	81.82
imipenem	4	36.36	4	36.36	3	27.27
Norfloxacin	11	100				
Pencillin G					11	100
Piperaclline	6	54.55	5	45.45		
Rifampicin	2	18.18			9	81.82
Vancomycin	2	18.18			9	81.82



DISCUSSION

In the eastern African countries, the prevalence of CLA on the endemicity level appears to be nearly similar **(Borham *et al.*, 2017).** The prevalence of CLA in camels reported in the current coincide with previous reports as 92 of 792 carcasses showed lesions (11.61%). The prevalence rates were 10 % in Ethiopia **(Domenech *et al.*, 1977),**10.9 % in Egypt **(Abou-Zaid *et al.*, 1994),** 12 % in Sudan **(Aljameel *et al.*, 2013a)** 10.35% in Egypt (**Borham *et al.*, (2017).** However, different prevalence rates were reported in Jordan by **Hawari (2008)** and in Saudi Arabia by **Radwan *et al.* (1989)** as their reported rates were 8% and 15%, respectively. Some cases in Saudi Arabia were characterized by multiple muscle and subcutaneous abscesses that may led to increased prevalence**.**

Live flocks and abattoir-based studies carried out in different parts of Egypt have shown that the data obtained for *C. pseudotuberculosis* prevalence vary.

Variations in the prevalence rates between the current study and others region Egypt may be attributed to the management system and climatic conditions in each region including the ambient temperature which affects the viability and spread of the bacteria

In this study, it was found that most of the internal lesion were detected in lung 64 (69.56 %). This prevalence is less than the incidence reported by **Awol *et al.*, (2011)** who reported 77.5 % lung lesions in camel carcasses in Ethiopia **and** higher than **Hamza *et al.* (2017)** in Sudan

whose reported lung lesions represented 51.4% of the internal lesions. The difference may be attributed to the adverse weather condition and accidental inhalation of biological organism (Bacteria and viruses) that may cause pneumonia as well as exposure to stress factors as dust and starvation which increase the probability of inhaled organism to cause damage and lesions in the lung **(Amen *et al.*, 2012; and Tenaw *et al.*, 2015).**

In the present study the incidence of liver lesion (4.35%) is slightly higher than those reported by **Al-ani *et al.* (1998)** in Jordan (1.2%) and **Nourani and Salimi, (2013)** in Iran (0.64%). However, **Aljameel *et al.* (2014) and Hamza *et al.*, 2107)** detected liver lesions in 13.5% and 45.7 % of the camel internal lesions, respectively. Such high incidence may be due to hepatocytes destruction induced by predisposing factors like liver flukes and toxic materials that have been absorbed from the gut enhancing formation of liver lesions and colonization of the lesions by opportunistic and pathogenic bacteria **(Scanlan and Edwards, 1990).**

In the current study, incidence of visceral form of CLA in camels was significantly more than the superficial form (8.71% versus 2.9 %). This contradicts the findings of **Borham *et al.* (2017)** who reported that superficial form of CLA in camels was more prevalent (9.76%) than the visceral form (0.58%). The difference may be attributed to the high spread of different microorganisms via inhalation or mucous membranes of the oral cavity damaged by dry and hard stems of desert plants. Additionally infection through wound directly or tick infestation or mange induced injuries are main predisposing factor for CLA **(Wernery and Kinne, 2016; Borham *et al.*, 2017).** In this study, visceral infection was found more prevalent in camels less than seven years old which agrees with what mentioned by **Aljameel *et al.* (2014)**. This finding suggests that the immune system of young camels is weaker than that of adults, which makes young camels more vulnerable to infection with pyogenic microorganism **(Devrajani *et al.*, 2010).** It was surprisingly observed 34.78% of camel lesions were bacteriologically negative when cultured on brain heart infusion agar. This can be attributed to the chronic nature of camel abscesses (nearly sterile) especially in the superficial form in which the organisms may be dead **(Zidan *et al.*, 2013)** or the abscesses might have been caused by viral, fungal, or parasitic agents **(Aljameel *et al.*, 2013 a)**.

All C. *pseudotuberculosis* isolates recovered in this study fermented trehalose and were catalase- and urease-positive which constitute a satisfactory basis for the identification of *C. pseudotuberculosis* **(Muckle and Gyles; 1982).**

It is well known that nitrate reduction test classifies C. *pseudotuberculosis* into two biovars, ovis (nitrate negative) and equi (nitrate positive) **(Costa *et al.*, 1998).** Interestingly, camel *C. pseudotuberculosis* isolates recovered in the current study all biovar equi on the basis of positive nitrate reduction. This support the results obtained by **Tejedor-Junco (2008)** who indicated that dromedary camels in Canaries Island were affected by serotype II (Var Equi). Meanwhile, **Berlin (2015)** and **Aljameel *et al.* (2013b)** typed *C. pseudotuberculosis* isolates from dromedaries as serotype I and serotype II. **Aljameel *et al.* (2013 b)** mentioned that infection with *C. pseudotuberculosis* serotype I or serotype II depends on husbandry practices, mixed herding, sharing of water and pastures, and migration with other animal species.

The antibiogram results revealed that the most effective antibiotic on the *C. pseudotuberculosis* was norfloxacin (100%). This agrees with the report of **Abebe and Tessema (2015)** in Ethiopia who reported that norfloxacin was highly effective on C. *pseudotuberculosis* isolates.

On the other hand, *C. pseudotuberculosis* isolates tested in this study were highly resistant to penicillin G (100%). This was mentioned earlier by **Hawarri (2008), Algammal (215)and Hamza (2017)**. The high level of resistance (81.82%) exhibited by C. *pseudotuberculosis* recovered in this study against gentamycin, rifampicin and vancomycin differs with the findings of **Muckle and Gyles (1982**) **and Hawari (2008)** who mentioned that all of their isolates were sensitive to gentamycin. These antibiotics are frequently used in veterinary and human medicine **(Teshome *et al.*, 2016).**

Some *C. pseudotuberculosis* isolates recovered in this study showed resistance to more than one out of the ten different antibiotics used. This suggests the existence of alarmingly multiple drug resistance of *C. pseudotuberculosis*.

The probable explanation to the presence of high antibiotic resistant *C. pseudotuberculosis* may be due to indiscriminate and repeated use of antibiotics regimes in animal and human health facilities **(Teshome *et al.*, 2016).** It was showed that normal flora /resident bacteria can harbor resistance genes to antibiotic (s). Transfer of resistance in bacteria has been documented to occur between different animal species **(Marshall *et al.*, (1990).**

Hence, attention is to be drawn towards the high prevalence of multidrug resistance among *C. pseudotuberculosis* isolates. Camel in markets and abattoirs and dealing with condemned organs represent a great risk for consumers and individuals in contact with animals or carcasses as multidrug resistant *C. pseudotuberculosis* may be transmitted to humans and cause disease **(Zunita *et al.*, 2008).** The exotoxin phospholipase D (PLD) is a major virulence factor in *C.*

pseudotuberculosis and thought to be so in *C. ulcerans* **(McNamara *et al.*, 1995; Dorella *et al.*, 2006).** The agent disseminates freely or within macrophages, mainly through the afferent lymphatic system, to local lymph nodes and internal organs **(Baty, 1986)**. This process depends on the ability of *C. pseudotuberculosis* to infect macrophages. PLD exerts its enzymatic effect that interrupts the normal function of ovine neutrophilchemotaxis.Consequently,bacteria resist phagolysosomes, inactivate complement, kill neutrophill, liberate new bacteria and cause necrosis ((**Yozwiak and Songer, 1993; Markey *et al.*, 2013).**

In addition, PLD acts on the phospholipids of mast cell membrane resulting in releasing of histamine- like substances such as leukotriene and prostaglandins**.** Moreover, PLD activates degranulation of cells resulting in liberation of many inflammatory mediators and cytokines. Such mediators, which are known to cause severe dilation and increased cell permeability followed by leakage of plasma and oedema **(Tizard, 1996; Cirino *et al.*, 1998).**

C. pseudotuberculosis isolates recovered in this study were subjected to PCR amplification using *pld* gene-specific primers. A 924 bp product was detected in all tested isolates, which agrees earlier findings of **(Goda *et al.*, 2007; Alharbi, 2011**; **Selim *et al.*, 2012; Syame *et al.*, 2013).** This confirms once more the major role in the pathogenesis of *C. pseudotuberculosis* and formation of the characteristic CLA lesions in different animals including camels.

Conflicts of interest:

All authors declare that they have no conflicts of interest.

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