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Highlighting on the occurrence of Corynebacterium pseudotuberculosis In camel

El-Gedawy, A .A*, Samah F. Ali*, Rasha A. Mohsen, Abeer E . Abd El Ghafar **, Yasser Hana***

*Bacteriology Dept., **Bacteriology Dep., Animal Health Research Institute, Mansoura branch, Agricultural Research Center

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ABSRTACT

Out of the 850 examined animals at Basattin abattoir, 88(10.35%) camels were affected by CLA. 83 animals had a detectable superficial lesions and only five animals had showed visceral lesions. The disease appeared clinically as superficial abscesses which varied in size from lemon to orange size or even larger containing pus of different colors (whitish creamy or greenish) and consistency (milk like, thick cheesy or dry firm). Most of the abscesses were closed and single, but occasionally opened and multiple. Large scars with ulcerated skin were formed in some cases especially at the inferior cervical and prescapular LNs. Visceral lesions were rare and only represented 0.58% of examined animals. Lungs, bronchial and mediastinal LNs were the detected organs which had visceral lesions containing caseous abscesses of different sizes, colors and consistency. Some affected LNs were congested and enlarged without abscess formation. Pus samples were collected aseptically for bacteriological isolation. Gram stained smears revealed gram positive non-spore forming bacilli and cocci shaped bacteria. Identification scheme of the gram positive non-spore forming rods was based on 3 successive steps; PCR, API Coryne system and Vitek2 system. Two C. pseudotuberculosis isolates were identified in addition to other coryneform bacteria as C. jeikeium and C. urealyticum. In addition, other bacteria such as cellulomonas spp./Microbacterium spp. and Brevibacterium spp. were also isolated.
Serum samples were collected from 93 camels and tested by exotoxin and SWC ELISAs. Accordingly, seropositivity percentage was reported to be 58.06% for exotoxin ELISA and 61.29% for SWC ELISA. The total seropositive camels were 77 animals by the two ELISAs with a seropositivity percentage of 82.79% compared to 35.4% of animals which showed a detectable clinical signs. The validity of bovine tuberculosis γ-IFN assay to detect cellular immunity against CLA in camels was detected. Although it was reported to be valuable to diagnose the disease in sheep

*Corresponding author: El-Gedawy, A .A, Bacteriology Dept, Animal Health Research Institute, Mansoura branch, Agriculture Research Center (ARC)
E-mail address: Dr.attia31@yahoo.com
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and goat, but this study showed that it was of no value in camels. Consequently, there is no cross reaction between bovine and cameloid γ-IFN monoclonal antibodies.

INTRODUCTION :

Camels are an important source for meat, milk, fiber, leather, and have a significance in the pastoral economy and transportation in tropical and subtropical regions (**Fowler, 2010**) Although camels are tolerant animals, but they are susceptible to many infectious diseases which have economic and zoonotic importance. Caseous lymphadenitis (CLA) is considered one of the most important bacterial diseases of camels **(Dioli, 2014; Mubarak and Moussa, 2022).** The disease has negative economic effects on camel production and trading which are represented in progressive weight loss of the affected animal in addition to carcass trimmings and skin condemnation at abattoirs.

CLA has superficial and visceral forms which may show detectable signs or remain subclinical. Clinical signs appear as abs cessation of the superficial lymph nodes (LNs), chronic weight loss or other symptoms according to the visceral organs affected **(Hawari, 2008; Silva et al. 2019).** The disease sometimes causes pneumonia, hepatitis, mastitis, arthritis, orchitis and meningitis. However, the visceral involvement is rare in camels **(Mubarak and Moussa, 2022; Wernery, 2012).**

Moreover, the onion ring pattern of the abscess which is common in
sheep and goat has been rarely described in camel LN and liver .

Diagnosis of CLA depends on clinical signs and characteristic post-mortem lesions (PM), but the confirmation is usually dependent on the isolation and identification of the causative agents. Other techniques such as PCR had been used for the detection of C. pseudotuberculosis in pus or its identification after initial culture (**Ilhan, 2013**). Bovine tuberculosis gamma interferon assay (γ-IFN) had been used successfully to diagnose CLA in small ruminants depending on cross reactivity between bovine, ovine and caprine γ-IFN monoclonal antibodies **(Paule et al. 2003).**

The aim of this study was Determination of the percentage of CLA in a representative sample of camels slaughtered for meat consumption atl-Basattine abattoir, Giza (Egypt) during the period from September 2020 to November 2022

Description of the clinical symptoms associated with CLA in camels.

Detection and characterization of visceral CLA lesions in camels.

Investigation of the bacteriological, molecular and serological aspects associated with CLA in camels.

Test the validity of bovine tuberculosis γ -IFN assay to diagnose CLA in camels which is not reported previously

MATERIAL and METHODS :

A total of 850 adult one humped camels (Camelus dromedarius) Animals:

for meat consumption , slaughtered at Basattin abattoir, were subjected to
clinical and P.M examination for CLA

Samples for bacteriological examination:

A total of 88 suspected lesions were aseptically collected from 88 animals; 75 abscessiated superficial LNs, 3 congested and swollen superficial LNs, 5 abscesses involving both superficial LNs with their overlaying skin, 2 visceral LN lesions and 3 lung abscesses.

Samples for ELISA

A total of 93 plain blood samples of affected (No. =33) and apparently normal (No. =60) camels slaughtered

Samples for γ-IFN assay:

Heparinized whole blood samples were aseptically collected from four CLA affected camels

Bacteriological examination:

Isolation and Identification of the causative organisms:
according to **(Tejedor et al. 2004).**

Analytical Profile Index (API) Coryne system:

The API Coryne system kit (bioMérieux, Marcy-l'Etoile, France) used for the identification of the isolated gram positive none-spore forming rods which were not identified by PCR targeting Pld of C. pseudotuberculosis

Vitek 2 compact system:

The vitek 2 system is an automated bacterial identification testing system that uses fluorescence based technology. This system used for identification of the gram positive none-spore forming rods which were not identified by API Coryne system according to (Biomerieux user guide, **(2006, France)..**

serological investigation :

Using indirect ELISA
Bradford method (Bradford, 1976).According to:

γ-IFN assay

γ-IFN assay for bovine tuberculosis**;** BOVIGAM®2G (PRIONICS AG, Switzerland) was used to diagnose CLA in four positive camels using the exotoxin antigen.

Molecular identification

Table 1. Primer sequences used in PCR:

Primer	Target gene	Sequence (5'-3')	Amplified product	Reference
Pld-F	Pld	ATA AGC GTA AGC AGG GAG CA	203 bp	(Ilhan, 2013)
Pld-R2		ATC AGC GGT GAT TGT CTT CCA GG		

Table 2. PCR reaction components.

Component	Volume/reaction
Emerald Amp GT PCR mastermix (2x premix)	12.5 µl
Pld-F (20 pmol)	1 µl
Pld-R2 (20 pmol)	1 µl
Bacterial DNA	6 µl
PCR grade water	4.5 µl
Total volume	25 µl

PCR conditions:

Cycling was performed under the following conditions ; initial heating at 94°C for 5 min. followed by 35 cycles of 94°C for 30 s, 56°C for 30 s and 72°C for 30 s. The final extension was at 72°C for 5 min.

Statistical analysis:

Chi-square was performed using SPSS 21

RESULTS:

Percentage of CLA in Bassatin abattoir examined camels:

Out of the 850 examined slaughtered camels at Bassatin abattoir, 88 (10.35%) were found affected by CLA. Superficial and visceral forms had been detected. However, the superficial form was more common than visceral form as illustrated in Table (3) and Figure (1).

Table 3. Percentage of CLA in camels at Bassatin abattoir during 2020-2022.

Form	No. of examined animals	No. of affected animals	Percentage
Superficial	850	83	9.76%
Visceral		5	0.58%
Total		88	10.35%

Superficial form is significantly ($p < 0.00001$) prevalent than visceral form.

Clinical findings:

Out of the 850 examined animals at Bassatin abattoir, 83 were affected by superficial form of CLA. The affected animals had showed enlargement of different superficial LNs with a distinct variation in size ranging from small lemon up to orange size or even larger

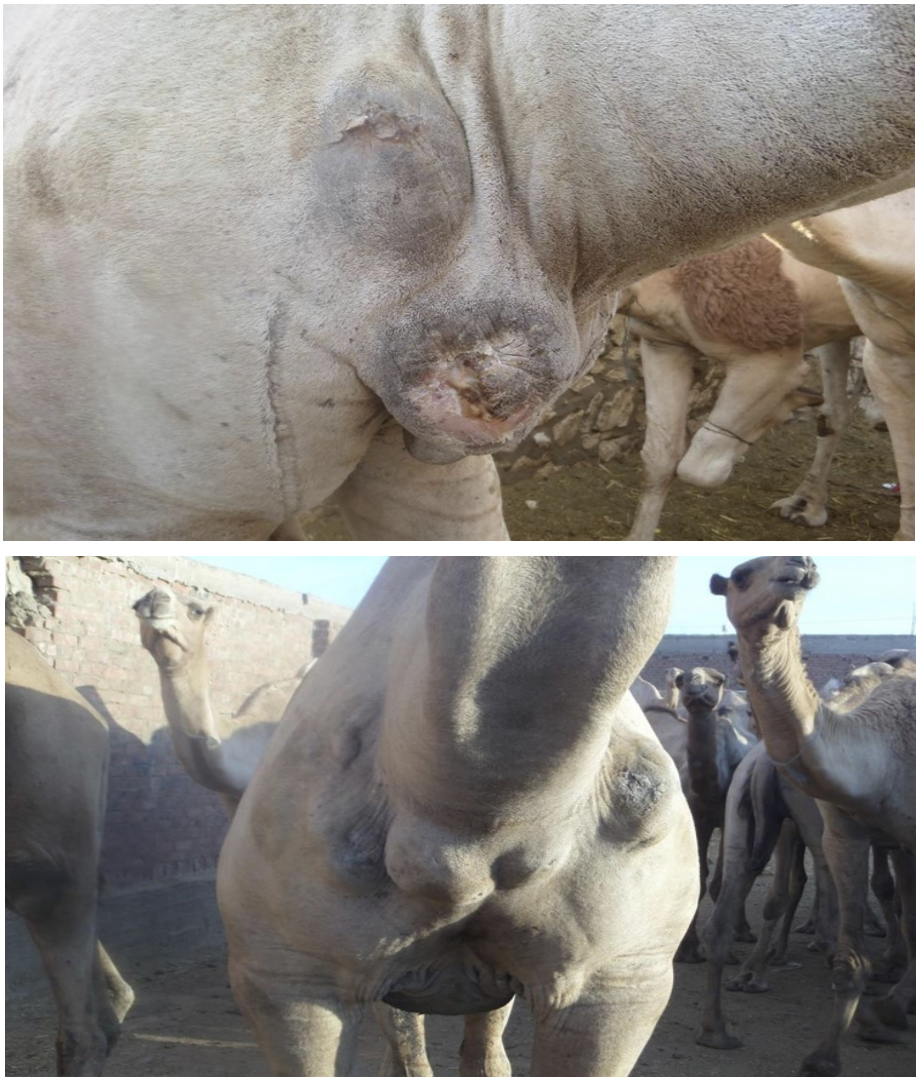


Fig. (1): CLA in camel involving both inferior cervical and both prescapular LNs with ulceration of outer skin .

Post mortem findings:

Out of the 850 animals examined at abattoir, only 5(0.58%) camels had showed a detectable PM lesions. Lungs, bronchial and mediastinal LNs were the detected organs which had visceral lesions .

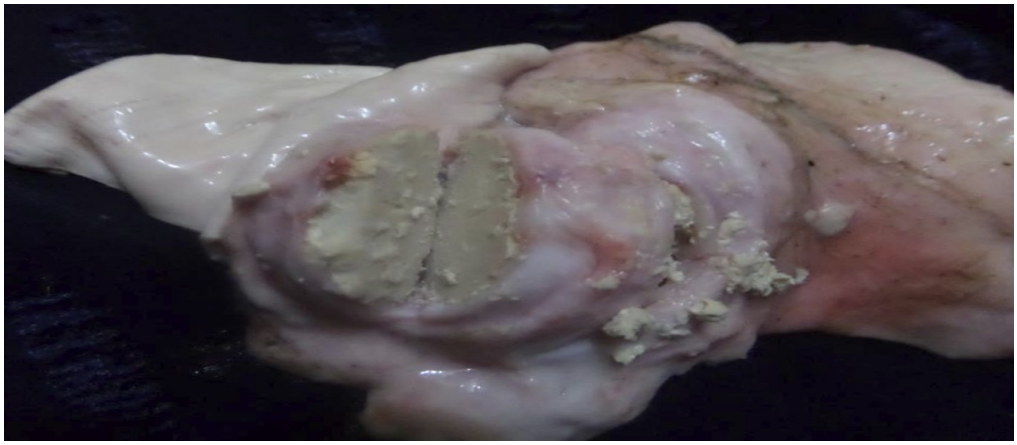


Fig 2. Incised bronchial LN showing dry firm greenish pus.

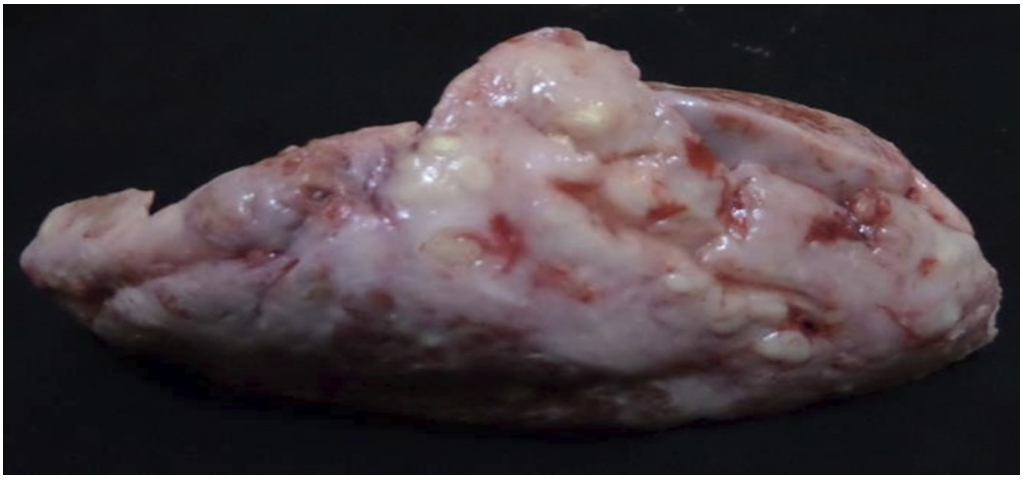


Fig 3. Pulmonary CLA lesion showing multiple small caseous abscesses.

Bacteriological findings and identification scheme of the isolated bacteria associated with CLA: Different bacteria had been isolated from 40.9% of the examined CLA cases . On the other hand, 59.1% (52 out of 88) of the cases did not yield any isolates.

Table (4): Different bacteriological isolates based on its shape as revealed by Gram stain

Type of isolate	No. of isolates (N=36)	% of total No. of isolates	% of total No. Of affected animal (N=88)
Gram positive non spore forming rods	11	30.55%	12.5%
Gram positive non spore forming rods +Gram positive cocci	4	11.11%	4.54%
Gram positive cocci	21	58.33%	23.86%

The identification scheme of the isolated Gram positive non-spore forming rods was based on three successive steps.

Firstly, selected 11 isolates were identified using PCR, targeting PLD gene of C. pseudotuberculosis. Out of them, two were identified as C. pseudotuberculosis biotype 1 (Figure 24).

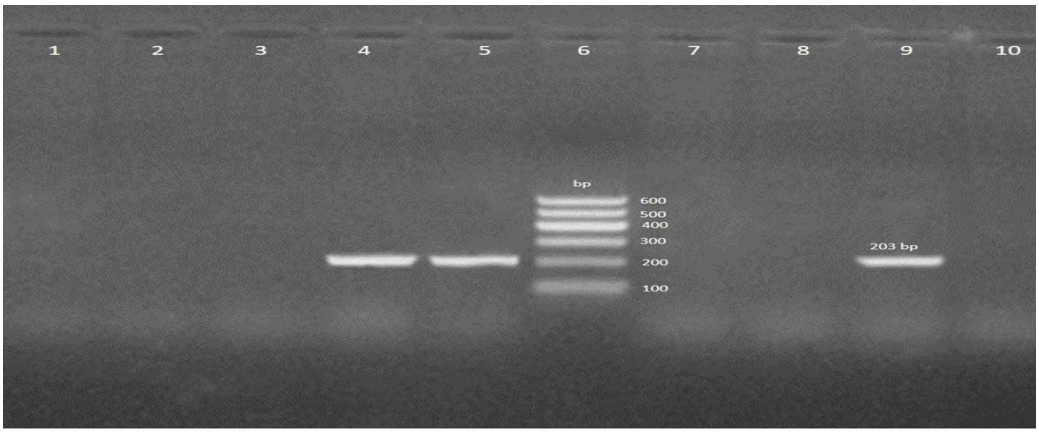
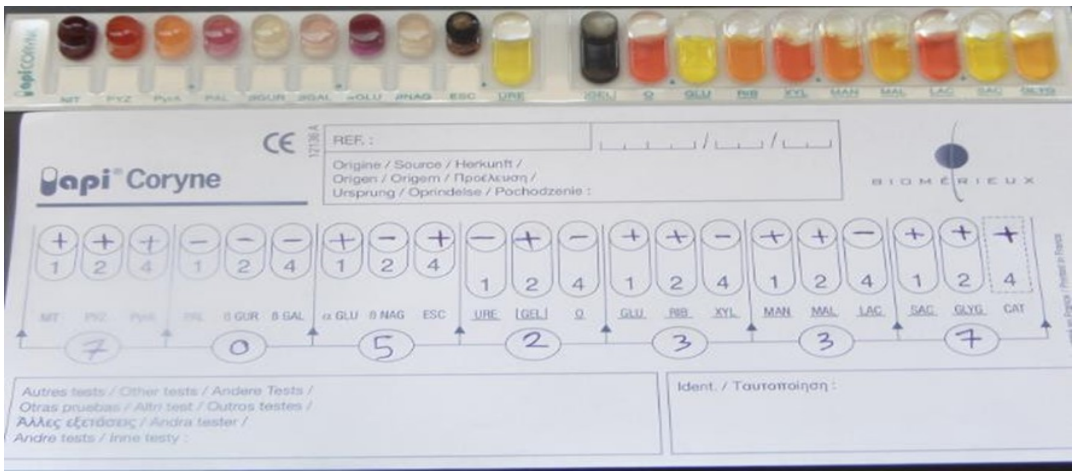


Fig. (4): PCR targetting 203 bp of C. pseudotuberculosis PLD gene; lane 6:DNA ladder ,lane 5 control positive, lane 10: control negative, lane 4 and 9: positive isolates and lane 1, 2, 3, 7 and 8: negative isolates.

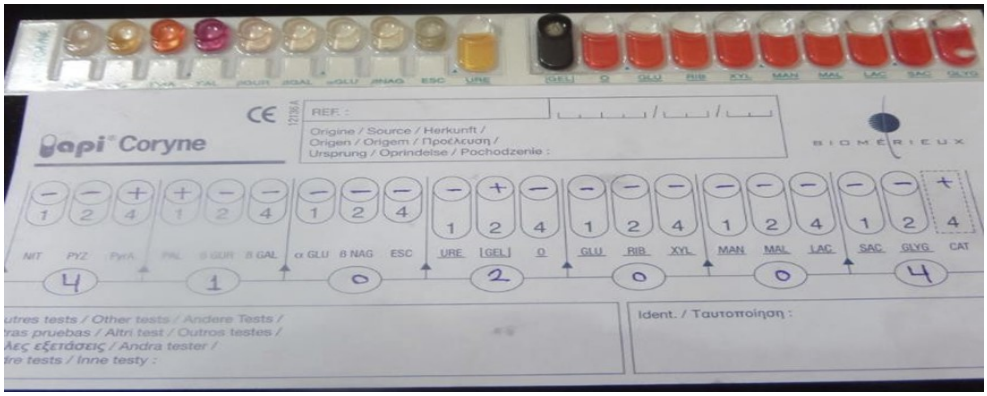
Second :The nine non-C.pseudotuberculosis Gram positive non-spore forming rods were tested by API Coryne system to be identified. Four out of these nine isolates were identified as Cellulomonas spp./ Microbacterium spp. (3 isolates have 3 different API profiles; 7652775, 3540775 and 7052337) and Brevibacterium spp. (1 isolate has 4102004 APIprofile)



Identification Information							
	Card:	ANC	Lot Number:	244366910	Expires:	Dec 30, 2016 12:00 CST
	Completed:	Feb 8, 2016 17:35 CST	Status:	Final	Analysis Time:	6.00 hours
Selected Organism	97% Probability		Corynebacterium urealyticum			
	Bionumber:	2200020400001			Confidence:	Excellent identification

Biochemical Details																			
4	dGAL	-	5	LeuA	+	6	ELLM	-	7	PheA	-	8	ProA	+	10	PyrA	-		
11	dCEL	-	13	TyrA	-	15	APPA	-	18	dGLU	-	20	dMNE	-	22	dMAL	-		
28	SAC	-	30	ARB	-	33	NAG	-	34	BGLUi	-	36	URE	+	37	BGURi	-		
39	BGALi	-	41	AARA	-	42	AGALi	-	43	BMAN	-	44	ARG	-	45	PVATE	+		
51	MTE	-	53	ESC	-	54	BdFUC	-	55	BNAGi	-	56	AMANi	-	57	AIFUC	-		
59	PHOS	-	60	IARA	-	61	dRIB2	-	62	OPS	-	63	AARAF	-	64	dXYL	-		
	GRAM	+		MORPH	?		AERO	?											

Fig 5 API results of Cellulomonas spp. /Microbacterium spp. showing 7052337 profile



GOOD IDENTIFICATION	
Strip	API CORYNE V3.0
Profile	4 1 0 2 0 0 4
Note	

Significant taxa	% ID	T	Tests against			
Brevibacterium spp	97.2	0.9				

Next taxon	% ID	T	Tests against			
Rhodococcus spp	1.1	0.47	PYRA 13%	αGLU 86%	GEL 6%	

Complementary test(s)	42°C			
Brevibacterium casei	-			
Brevibacterium epidermidis	+(-)			

Fig 6. API result of Brevibacterium spp. showing 4102004 profile number.

Third :Out of the five API unidentified isolates, two were identified as C. jeikeium and C. urealyticum with a very good and excellent identification confidence, respectively .

Identification Information	Card:	ANC	Lot Number:	244366910	Expires:	Dec 30, 2016 12:00 CST
	Completed:	Feb 8, 2016 17:35 CST	Status:	Final	Analysis Time:	6.00 hours
Selected Organism	97% Probability		Corynebacterium urealyticum			
	Bionumber:	2200020400001			Confidence:	Excellent identification

Biochemical Details																			
4	dGAL	-	5	LeuA	+	6	ELLM	-	7	PheA	-	8	ProA	+	10	PyrA	-		
11	dCEL	-	13	TyrA	-	15	APPA	-	18	dGLU	-	20	dMNE	-	22	dMAL	-		
28	SAC	-	30	ARB	-	33	NAG	-	34	BGLUi	-	36	URE	+	37	BGURi	-		
39	BGALi	-	41	AARA	-	42	AGALi	-	43	BMAN	-	44	ARG	-	45	PVATE	+		
51	MTE	-	53	ESC	-	54	BdFUC	-	55	BNAGi	-	56	AMANi	-	57	AlFUC	-		
59	PHOS	-	60	lARA	-	61	dRIB2	-	62	OPS	-	63	AARAF	-	64	dXYL	-		
	GRAM	+		MORPH	?		AERO	?											

Fig 7. The print out sheet of Vitek2 compact system showing the collection of biochemical reactions of C. jeikeium.

Identification Information	Card:	ANC	Lot Number:	244366910	Expires:	Dec 30, 2016 12:00 CST
	Completed:	Jan 4, 2016 17:37 CST	Status:	Final	Analysis Time:	6.00 hours
Selected Organism	94% Probability		Corynebacterium jeikeium			
	Bionumber:	6103000400001			Confidence:	Very good identification

Biochemical Details																			
4	dGAL	-	5	LeuA	+	6	ELLM	+	7	PheA	+	8	ProA	-	10	PyrA	-		
11	dCEL	-	13	TyrA	-	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	-		
28	SAC	-	30	ARB	-	33	NAG	-	34	BGLUi	-	36	URE	-	37	BGURi	-		
39	BGALi	-	41	AARA	-	42	AGALi	-	43	BMAN	-	44	ARG	-	45	PVATE	+		
51	MTE	(-)	53	ESC	-	54	BdFUC	-	55	BNAGi	-	56	AMANi	-	57	AlFUC	-		
59	PHOS	-	60	lARA	-	61	dRIB2	-	62	OPS	-	63	AARAF	-	64	dXYL	-		
	GRAM	+		MORPH	?		AERO	?											

Fig 8. The print out sheet of Vitek2 compact system showing the collection of biochemical reactions of C. urealyticum.

Table 5. Results of identification scheme of the isolated Gram positive non spore forming rods.

Isolate type	No. of isolates	Method of identification
C. pseudotuberculosis	2	PCR
Cellulomonas spp./Microbacterium spp.	3	API Coryne system
Brevibacterium spp.	1	API Coryne system
C. jeikeium	1	Vitek2 system
C. urealyticum	1	Vitek2 system
Unidentified	3	-

ELISA test results:

Table 6. Reactivity of affected and apparently normal camels to ELISA testing

Type of antigen	Clinical status of tested animals	No. of samples	ELISA results			
			Positive		Negative	
			No.	%	No.	%
Exotoxin	Affected	33	26	78.78%	7	21.21%
	Apparent healthy	60	28	46.66%	32	53.33%
	Total	93	54	58.06%	39	41.93%
SWC	Affected	33	24	72.72%	9	27.27%
	Apparent healthy	60	33	55%	27	45%
	Total	93	57	61.29%	36	38.71%

γ-IFN assay results:

Table 7. OD values of γ-IFN assay tested camels in relation to increased exotoxin antigen concentration.

Animal	OD of blank sample	OD at different concentrations of antigen				
		5µg	10µg	20µg	40µg	80µg
1	0.126	0.201	0.148	0.129	0.150	0.154
2	0.100	0.169	0.157	0.210	0.136	0.122
3	0.007	0.133	0.100	0.089	0.090	0.112
4	0.077	0.055	0.093	0.078	0.076	0.081

DISSCUSSION

Caseous lymphadenitis is a worldwide infectious disease of camels

The disease also affects other species such as sheep, goat, pigs, deer and many wild animals **(de Sá Guimarães et al. 2011).** The disease express its self in two forms, external form in the superficial LNs and internal form (located in visceral LNs and internal organs especially the lung, liver, mediastinal and bronchial LNs) **(Hawari, 2008).** The percentage of CLA among the abattoir examined camels was 10.35 % .Nearly similar results were reported by **(Abou-Zaid et al., 1994; Mubarak and Moussa, 2022).**

The superficial form (9.76%) was significantly more prevalent than the visceral form (0.58%).Nearly similar findings were recorded by **(Silva et al. 2019; Wernery, 2012).**

CLA lesions were of variable size ranging from lemon to orange size or even larger. The size of CLA lesion is dependent on the stage of the disease, potency and amount of the bacterial exotoxin in addition to the immune status of the animal **(Mubarak and Moussa, 2022; Wernery, 2012).**

The majority of CLA lesions were cold, hard and painless and occasionally, they had showed ulceration and scar formation. Similar findings were reported by **(Domenech et al. 1977);** Concerning the number of superficial lesions per animal; 81.68%,13,74 % and 4.58% of the affected cases had showed a single, double and multiple lesions, respectively. Similarly, **(Siddiqui and Telfah, 2010).**

The isolation rate represented 40.9% of the examined cases while 59.1 % of cases did not yield growth. Isolation failure in some cases is mainly due to nature of lesions which mostly is old lesions containing low numbers of viable organisms and become nearly sterile **(Oreiby, 2013).**

The most predominant species isolated were pure Gram positive cocci (33.55%) followed by pure Gram positive non spore forming rods (35.33%) and finally mixed Gram positive non spore forming rodswith Gram positive cocci (11.11%). Two isolates were identified by PCR as C. pseudotuberculosis out of 11. The lower isolation rate of C. pseudotuberculosis may be attributed to the presence of other bacteria such as Staphylococci and Streptococci that overshadow sensitive C. pseudotuberculosis on culture media **(Mubarak et al. 1999)** or due to sampling from a sterile part of the abscess **(Brown et al. 1987).**

Similarly, **Zidan et al. (2013**) had isolated two C. pseudotuberculosis out of16 Corynebacterium spp. isolates from camels.

Other isolated non-C. pseudotuberculosis gram positive non-spore forming were identified as Cellulomonas spp. / Microbacterium spp. (3 isolates) and Brevibacterium spp. (1 isolate) using API Coryne system. Furthermore, one C. jeikeium and one C. urealyticum isolates wereidentified by Vitek2 system. The pathogen C. urealyticum was previously isolated from CLA lesion of sheep by **Huerta et al. (2013)** who identified it using 16S rRNA sequencing, but failed to be identified by two different biochemical methods (API CS and BPCS). In this study C. urealyticum and C. jeikeium were identified by Vitek2 system while the API Coryne system failed to identify them. This explains that there is a difference in the sensitivity of identification between the two methods.

Exotoxinand SWC antigens based ELISA tests revealed seropositivity of 58.06%and 61.29%, respectively.

Higher seropositivity percentage of SWC ELISA than exotoxin- ELISA is mainly due to wider cross reactivity between somatic antigens of the causative bacteria than that of their exotoxin antigens. The total seropositivity of tested animals was 82.79% using different ELISAs. This high seroprevalence indicates that there is a huge silent infection among imported camels con-

sequently, large economic losses due to the wide spread of infection. So, subclinical infection should be considered becausethey allow dissemination of infection. Clinical and postmortem examinations of the serologically tested 93 camels had showed lesions in 35.4% of camels compared to seropositivity percentage of 82.79% by the two ELISAs. Consequently, depending only on the detection of visible lesions in live and slaughtered camels will yield an underestimation of the disease. This is mainly due to subclinical cases or animals with small non-progressive lesions (may reach millimeters in size) which may escape postmortem examination. Although bovine γ-IFN assay had been used successfully to diagnose CLA in sheep and goat **(Paule et al. 2003 Prescott et al. 2002),** but it was of no value in camels as there was no change of OD values of four CLA confirmed cases upon the increased concentration of the stimulating antigen. Consequently, there is no cross reaction between bovine and cameloid γ-IFN monoclonal antibodies.

CONCLUSSION

CLA is a prevalent disease among Sudanese and Somali imported camels for meat consumption in Egypt.

CLA in camels has a chronic nature which may appear as congestion, abscessiation and scar formation.

Visceral CLA lesions are rare in the examined camels.

Corynebacterium pseudotuberculosis, C. jeikeium, C. urealyticum, Cellulomonas spp. / Microbacterium spp., Brevibacterium spp. and other gram positive cocci are associated with CLA in camels.

Serological testing revealed a high seropositivity among the examined imported camels (82.7%).

On the contrary, the absence of a detectable lesion does not indicate seronegativity of the tested animal.

Bovine γ-IFN assay has no value to diagnose CLA in camels.

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