



## SHEEP CASEOUS LYMPHADENITIS: CAUSATIVE BACTERIAL AGENTS AND THEIR IN VITRO SOME ANTIMICROBIAL SUSCEPTIBILITY

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### ABSTRACT:

*Corynebacterium pseudotuberculosis* (*C. pseudotuberculosis*) is the etiologic agent of caseous lymphadenitis. It is a serious, economically important disease for sheep production. This study was carried out on 762 sheep reared in private flocks (616 females and 146 males) with different ages at different localities in Assiut Governorate. The prevalence of the disease in sheep was 22.7% on the basis of clinical signs and 20.1% on the basis of bacteriological examination. The disease prevalence was significantly higher in females' sheep (25.3%) than that in males (11.6%). Caseous lymphadenitis was highly significantly different among age groups. The high prevalence of caseous lymphadenitis was recorded in sheep of 1-2 years old (37.34%), followed by animals of 2-3 years old (25.77%) and the low prevalence was in sheep under one year old (8.33%), this may be due to occurrence of maternal antibodies from colostrum. Parotid lymph nodes were the most common affected lymph node (11.81%), followed by superficial cervical lymph nodes (7.87%), while prefemoral lymph node was less affected (0.39%). The superficial lymph nodes of the anterior body half showed the highest rate of infection than the posterior body half. *C. pseudotuberculosis* was the main causative agent of caseous lymphadenitis in sheep, where it was isolated either in pure form or in combination with *Staph. aureus* from 142 cases (82.1%) and 7 cases (4%) of the clinically infected sheep, respectively. On the other hand *Staph. aureus* was isolated as a sole pathogen from 4 cases representing 2.3%. All isolated strains of *C. pseudotuberculosis* were highly virulent to Guinea pigs and 97.3% of them were nitrate reduction negative. *In vitro* antimicrobial susceptibility test of *C. pseudotuberculosis* strains showed that Enrofloxacin and Ciprofloxacin had strong inhibitory effect on the isolates, while the most strains were highly resistant to Lincomycin, Streptomycin, Amoxycillin and Penicillin. From the obtained results, it can be concluded that *C. pseudotuberculosis* is the main causative agent of caseous lymphadenitis in sheep. The superficial lymph nodes of the anterior body half show the highest rate of infection than the posterior body half. Enrofloxacin and Ciprofloxacin are the most effective drugs during *in vitro* tests.

### INTRODUCTION:

Caseous lymphadenitis is a worldwide chronic infectious disease of small ruminants characterized by formation of pyogranulomas mainly in superficial lymph nodes and rarely in visceral lymph nodes and organs (Baird and Fontaine, 2007). Caseous lymphadenitis caused

by *Corynebacterium pseudotuberculosis* (*C. pseudotuberculosis*), which can be transmitted by contamination of skin wounds, but can also be acquired by penetration of intact skin, ingestion and probably through inhalation (Radostits *et al.*, 2007). Shearing and abrasions appeared to play an important role in spreading of the

disease among animals (Al-Gaabary *et al.*, 2010). The presence of abscesses in superficial lymph nodes is highly suggestive for caseous lymphadenitis particularly if several animals in a group are similarly affected. Confirmatory diagnosis of the disease is mainly based on culture and identification of *C. pseudotuberculosis* (Baird and Fontaine, 2007).

Economic losses resulting from caseous lymphadenitis are reflected in carcass condemnation, decline in wool production and death of severely affected sheep and perhaps decreased reproductive performance resulting from thin ewe syndrome. These economic losses are so severe in the disseminated visceral form due to extensive internal abscesses (Standford *et al.*, 1998; Baird and Fontaine, 2007). Also, caseous lymphadenitis can become a public health problem as it is a zoonosis (Jain- Lambert *et al.*, 2006).

The aim of the present study was carried out to estimate the prevalence of caseous lymphadenitis in sheep and the distribution of the lesions in superficial lymph nodes and to investigate the bacterial agents causing the disease. Also to study the virulence and pathogenicity to Guinea-pigs as well as *in vitro* antibiogram to the local isolates.

## MATERIAL AND METHODS:

### Field Animals:

A total of 762 sheep reared in private flocks (616 female and 146 male) of different ages, at different localities in Assiut Governorate, were subjected to clinical examination with special attention to superficial lymph nodes and bacteriological examination for caseous lymphadenitis.

### Bacteriological examination:

The samples from each lesion were collected either by aspiration from closed lesions or via cotton swabs from the pyogenic membrane of the opened lesions. All samples were taken under complete aseptic condition and used for isolation of the causative agent. The samples were seen in 10% serum broth for 24 hours at 37° as this procedure resulted in enrichment of culture and made primary isolation much easier (Mostafa, *et al.*, 1973), then subculture on 10% sheep blood agar, MacConkey's agar which incubated at 37° for 24 hours. The characteristic colonial appearance of *Corynebacterium pseudotuberculosis* on blood agar plates were selected and transferred to slants of nutrient agar and incubated at 37° for 24 hours. The resulting colonies were subjected to catalase test, Gram's staining, nitrate reduction, urease, gelatin liquefaction test, as well as fermentation of the following sugars: glucose, sucrose and maltose according to the method described by Quinn *et al.* (1994).

### Experimental animals' inoculation:

Ten male Guinea pigs about 250-350 g. body weight were used for determination of its pathogenicity as well as reisolation of *C. pseudotuberculosis*. Eight of them were injected intramuscularly with overnight incubated culture broth of the isolated *C. pseudotuberculosis* in the hind limb with single dose of 1 ml bacterial ( $10^{13}$  CFU/ml) according to El-Far (1976). The other two male Guinea pigs were injected with normal saline (control negative). The injected Guinea pigs were checked daily up to 9 days



and reisolations of the inoculated strain were done from lesions.

### Antibiogram:

Antibiogram of 54 recovered isolates of *C. pseudotuberculosis* were tested using antimicrobial susceptibility testing by disc diffusion standard technique according to Quinn *et al.* (1994). The isolated strains were tested against 13 antimicrobial agents (Amoxicillin 10 µg, Cefotaxime 30 µg, Cephalexin 30 µg, Ciprofloxacin 5 µg, Doxycycline 30 µg, Enrofloxacin 5 µg, Gentamicin 10 µg, Lincomycin 2 µg, Neomycin 30 µg, Oxytetracycline 30 µg, Penicillin 10 U, Streptomycin 10 µg and Trimethoprim-Sulfamethoxazole 25 µg), (Bioanalyse-Turkey). Statistical analysis was done using Chi-square by SPSS, 2005 program (Statistical Package for Social Sciences for Windows Release 14.0.0.).

### RESULTS:

Detailed obtained results were illustrated in Tables (1-5).

### Clinical signs:

The clinical signs of caseous lymphadenitis were in the form of enlargement of one or more superficial lymph node and abscessation of affected lymph nodes which either opened or closed (Figs. 1& 2) and the wool over the lesions was lost in some cases (Fig. 3).

### Pathogenicity:

Two inoculated Guinea pigs with overnight incubated culture broth of the isolated *C. pseudotuberculosis* died within 48 hours. Oedema at the site of the inoculation and internal organs and no abscess was developed. The other six Guinea pigs died within 2-5 days, these injected Guinea pigs showed abscesses at site of inoculation and congestion of internal organs and small abscesses in liver and spleen. *C. pseudotuberculosis* reisolated from the site of inoculation, preformal lymph node and abscesses in the internal organs. The two control negative Guinea pigs were not affected.

Table 1 : The prevalence of the caseous lymphadenitis in the examined sheep

Examined animals	Sex**	Clinically affected sheep		Bacteriological +ve from affected sheep		Bacteriological +ve from examined sheep	
		No.	%	No.	%	No.	%
Sheep (N=762)	♀ (N= 616)	156	25.3	141	90.4	141	22.9
	♂ (N=146)	17	11.6	12	70.6	12	8.2
Total	762	173	22.7	153	88.4	153	20.1

\*\*Highly significant statistical variations

$\chi^2 = 12.588$

$p < 0.001$

**Table 2 : Prevalence of caseous lymphadenitis in relation to age in the examined sheep**

Age group**	No. of the examined	Infected sheep	
		No.	%
< 1 year old	72	6	8.33
1-2 year old	241	90	37.34
2-3 year old	163	42	25.77
3-4 year old	137	22	16.06
> 4 year old	149	13	8.72
<b>Total</b>	<b>762</b>	<b>173</b>	<b>22.70</b>

\*\*Highly significant statistical variations  $\chi^2 = 58.82$   $p < 0.0003$

**Table 3 : Distribution of the affected superficial lymph nodes in the examined sheep (No. = 762)**

Lymph node	Unilateral clinically affected		Unilateral bacteriologically positive		Bilateral clinically affected		Bilateral bacteriologically positive		Total affected lymph nodes in examined cases	
	No.	%	No.	%	No.	%	No.	%	No.	%
Parotid	85	11.16	77	10.11	5	0.66	3	0.39	90	11.81
Superficial cervical	58	7.61	51	6.69	2	0.26	2	0.26	60	7.87
Mandibular	20	2.63	17	2.23	0	0	0	0	20	2.63
Prefemoral	3	0.93	3	0.39	0	0	0	0	3	0.39
<b>Total</b>	<b>166</b>	<b>21.79</b>	<b>148</b>	<b>19.42</b>	<b>7</b>	<b>0.92</b>	<b>5</b>	<b>0.65</b>	<b>173</b>	<b>22.7</b>

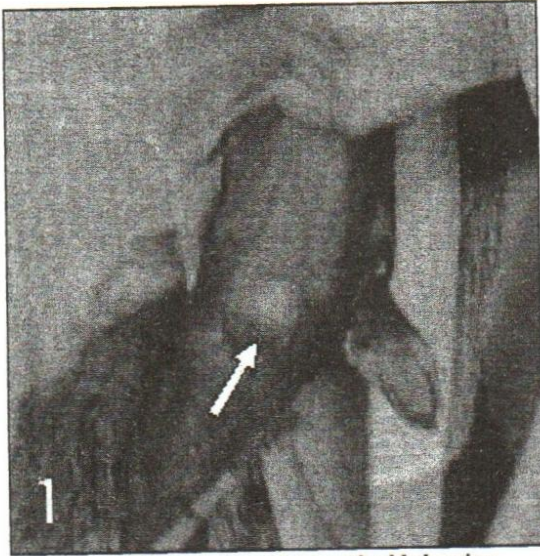
**Table 4 : Bacteriological examination and bacterial isolates from the clinically affected sheep (N =173)**

Bacteriological				Bacterial isolates				Nitrate reduction test of <i>C. pseudotuberculosis</i> (N=149)			
(+ve)		(-ve)		Pure forms		Mixed forms		(+ve)		(-ve)	
No.	%	No.	%					No.	%	No.	%
153	88.4	20	11.6	<i>C. pseudotuberculosis</i> 142 (82.1%) <i>Staph. Aureus</i> 4 (2.3%)		<i>C. pseudotuberculosis</i> with <i>Staph. Aureus</i> 7 (4%)		145	97.3	4	2.7

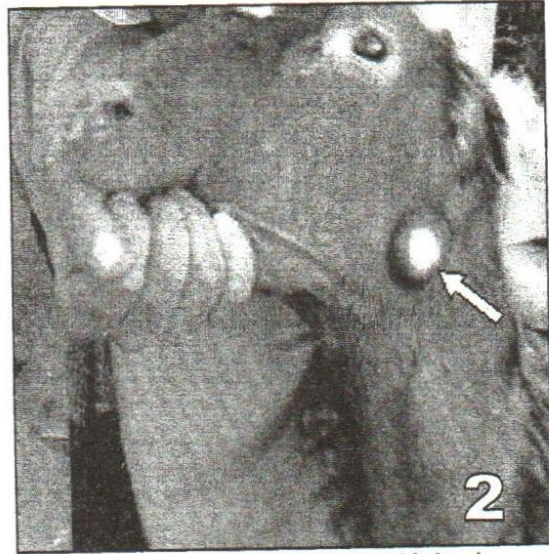
**Table 5 : In vitro antimicrobial sensitivity of 54 strains of *C. pseudotuberculosis* isolated from sheep affected with caseous lymphadenitis**

Antimicrobial agents	Conc. Per disc	Resistant		Intermediate		Susceptible	
		No.	%	No.	%	No.	%
Amoxicillin	10 µg	48	88.9	3	5.6	3	5.6
Cefotaxime	30 µg	39	72.2	3	5.6	12	22.2
Cephalexin	30 µg	39	72.2	6	11.1	9	16.7
Ciprofloxacin	5 µg	0	0	3	5.6	51	94.4
Doxycycline	30 µg	15	27.8	0	0	39	72.2
Enrofloxacin	5 µg	0	0	0	0	54	100
Gentamicin	10 µg	6	11.1	3	5.6	45	83.3
Lincomycin	2 µg	54	100	0	0	0	0
Neomycin	30 µg	15	27.8	0	0	39	72.2
Oxytetracycline	30 µg	18	33.3	0	0	36	66.7
Penicillin	10 U	33	61.1	15	27.8	6	11.1
Streptomycin	10 µg	49	90.7	0	0	5	9.3
Trimethoprim-Sulfamethoxazole	25 µg	33	61.1	3	5.6	18	33.3





**Fig. 1 : Male sheep eight month old showing enlargement of the mandibular lymph node infected with caseous lymphadenitis**



**Fig. 2 : Female sheep four years old showing enlargement of the parotid lymph node infected with caseous lymphadenitis**



**Fig. 3 : Male sheep one year old showing enlargement of the superficial cervical lymph node infected with caseous lymphadenitis, notice loss of wool over the affected area**

## DISCUSSION:

*Corynebacterium pseudotuberculosis* is the etiologic agent of caseous lymphadenitis, which is a serious, economically important problem for sheep production (Guimarães *et al.*, 2009).

Concerning the clinical picture of examined sheep, the disease was appeared in the form of enlargement of one or more superficial lymph node and abscessation of the affected superficial lymph nodes with loss of the wool over the lesion. Previous records were registered by Zaitoun and Ali (1999) and Al-Gaabary *et al.* (2009).

As shown in Table (1) the prevalence of caseous lymphadenitis in examined sheep was 22.7% on the basis of clinical signs and 20.1% on the basis of bacteriological examination. Similar rates were reported by Zaitoun and Ali (1999) 20%; Paton *et al.* (2003) 26% and Al-Gaabary *et al.* (2009) 23.33%. High rates (30.17 and 39.13%) were reported by Zaitoun and Bayoumi (1994) and Zaitoun *et al.* (2009), respectively, while low rates were reported by Mubarak *et al.* (1999) 6.78%; Maarouf and Enas (2008) 7.1% and Al-Gaabary and El-Sheikh (2002) 13.36%. The variations in the disease frequency between different studies may be attributed to the differences in the management systems and climatic conditions in each study where, the viability of the causative organism in the contaminated environment is greatly affected by ambient temperature. Also, it may be attributed to the endemic nature of the disease which leading to a variation in animal immunity or the degree of its susceptibility,

in addition to the varied locality or habitat in which animals live (Al-Gaabary *et al.*, 2009).

In the present work the prevalence of caseous lymphadenitis was significantly higher ( $P < 0.001$ ) in females sheep (25.3%) than that in males (11.6%), Table (1). Similar results were obtained by Nadim and Farid (1973) and Al-Gaabary *et al.* (2009). While Zaitoun and Ali (1999) mentioned that the male sheep were apparently more susceptible to *C. pseudotuberculosis* than females. On the other hand, the results obtained by Seddik *et al.* (1983) and Zaitoun and Bayoumi (1994) reported that there was no significant difference in the susceptibility of male and female sheep to *C. pseudotuberculosis*. This may ascribed to the unbalanced ratio between females and males sheep of the investigated flocks, the rams take more attention in the herds and the age of the most males in the present work was over three years old and build up of acquired immunity.

Concerning age predisposition in the present study, caseous lymphadenitis was highly significantly different ( $P < 0.0003$ ) among age groups, Table (2). Higher prevalence was recorded in the sheep of age group from 1-2 years old (37.34%), followed by animals of age group 2-3 years old and age group 3-4 years old (25.77 & 16.06%, respectively). The age group under one year old of examined sheep was the lowest rate (8.33%). Similar results were obtained by Zaitoun and Ali (1999); O' Reilly *et al.* (2008); Guimarães *et al.* (2009) and Al-Gaabary *et al.* (2009). On the contrary different results were recorded by Al-Gaabary and El-Sheikh (2002).



The obtained results may be attributed to the fact that the prevalence of caseous lymphadenitis increases along with the progress in animals age, where becoming older means more exposure to risk factors as shearing. Higher prevalence of the disease in age groups 1-2 and 2-3 years old was indicative of losing maternal immunity and long contact of these groups with pus material. Diminution of infection rate after four years may be ascribing to build up of acquired immunity, Zaitoun and Bayoumi (1994).

The lower prevalence of caseous lymphadenitis in sheep under one year old in the present work may due to maternal antibodies from colostrum, Al-Gaabary *et al.* (2009). The limited number of affected lambs can attributed to the fact that the disease usually progresses slowly and therefore the lesions are mainly detected in adult animals (Severini *et al.*, 2003).

In the present study the obtained results (Table, 3) revealed that parotid lymph node was the most common affected lymph node followed by superficial cervical lymph node, while pre-femoral lymph node was less affected. The superficial lymph node of the anterior body half was the most commonly affected with caseous lymphadenitis than the posterior body half. The same results were obtained by Mubarak *et al.* (1999) and Al-Gaabary *et al.* (2009). These results disagree with that obtained by Zaitoun and Bayoumi (1994); Abd El-Ghani *et al.* (1998) and Zaitoun and Ali (1999). Zaitoun *et al.* (2009) noticed that the most prominent enlarged lymph nodes of the examined sheep were prescapular lymph nodes. The highest percentage of parotid

lymph node infection give an indication that ingestion, shearing practices or head butting have a considerable role in transmission of caseous lymphadenitis in sheep (Al-Gaabary *et al.*, 2009).

In the present study bacteriological examination, Table (4) showing that out of 173 sheep, showing typical lesions of caseous lymphadenitis, 153 cases were positive for bacterial isolation representing 88.4% and *C. pseudotuberculosis* was isolated from 149 cases (86.13%) from the infected sheep. *C. pseudotuberculosis* was isolated either in pure form or in combination form with *Staph. aureus* from 142 cases (82.1%) and 7 cases (4%) of the infected sheep, respectively. While *Staph. aureus* was isolated as a sole pathogen from 4 cases representing 2.3%. The obtained results indicated that *C. pseudotuberculosis* is the main causative agents of caseous lymphadenitis in sheep. Similar reports were previously reported (Abd El-Ghani *et al.*, 1998; Basma *et al.*, 2003; Dorella *et al.*, 2006; Belchior *et al.*, 2007; Fontaine and Baird, 2008; Maarouf and Enas, 2008; and Zaitoun *et al.*, 2009). *C. pseudotuberculosis* was detected in 90.07% of clinically infected sheep (Al-Gaabary *et al.*, 2009). In present work failure for bacterial isolation was detected from 20 (11.6%) of the diseased sheep. Failure of bacterial isolation from infected sheep with caseous lymphadenitis was 5.5% (Zaitoun *et al.*, 2009). It is possible to isolate the organism from lesions of all cases affected with caseous lymphadenitis, although the number of viable bacteria present in chronic abscess may be low and apparently sterile le-

sions are occasionally encountered (Baird and Fontaine, 2007). The defense mechanism of *C. pseudotuberculosis* prevents the organism from being isolated in all bacteriology cultures, (Radostits *et al.* 2007).

*C. pseudotuberculosis* is grouped in two different biotypes (serotypes) which differ from each other biochemically, serologically and epidemiologically. The most common feature of variation is the ability of serotype I to reduce nitrate to nitrite and failure of serotype II to do that (Quinn *et al.*, 1994). *C. pseudotuberculosis* strains isolated from small ruminants generally do not reduce nitrate (Williamson, 2001 and Zaitoun *et al.*, 2009). In the present study 97.3% of the isolated strains of *C. pseudotuberculosis* were nitrate reduction negative, (Table 4).

Resistance of *C. pseudotuberculosis* to antibiotics and its strenuous perseverance in the environment, associate with difficulty in detecting infected animals, make caseous lymphadenitis hard to eradicate (Williamson, 2001). In present work *in vitro* antimicrobial sensitivity test of *C. pseudotuberculosis* strains isolated from infected sheep revealed that Enrofloxacin and Ciprofloxacin had strong inhibitory effect on the tested isolates (100% and 94.4%, respectively), Table (5). These results go in parallel with that obtained by Amany and Hala (2008). The most strains were highly resistant to Lincomycin, Streptomycin, Amoxicillin and Penicillin, Similar results mentioned by Basma, Shalaby *et al.* (2003). All isolated strains of *C. pseudotuberculosis* were Streptomycin resistant (Zaitoun and Ali, 1999). Garg *et al.* (1985) and

Pepin *et al.* (1989) reported strains of *C. pseudotuberculosis* were strongly resistant to Penicillin and Streptomycin and susceptible to Neomycin. On the other hand all tested strains of *C. pseudotuberculosis* were completely sensitive to Penicillin-G (Abd El-Ghani *et al.*, 1998), while it was highly sensitive to Amoxicillin, Rifamycin and Gentamicin (Maarouf and Enas, 2008).

Concerning to the pathogenicity and toxogenic action of the isolated *C. pseudotuberculosis* strains to Guinea pigs abscesses were developed either at the site of inoculation or paranchymatous organs, in addition to death of the all inoculated Guinea pigs within 2-5 days post inoculation. The same results were obtained by Dorella *et al.*, (2006) and Maarouf and Enas (2008).

From the obtained results, it can be concluded that *C. pseudotuberculosis* is the main causative agent of caseous lymphadenitis in sheep. The superficial lymph nodes of the anterior body half show the highest rate of infection than the posterior body half. Enrofloxacin and Ciprofloxacin are the most drugs of choice for caseous lymphadenitis.

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## مرض السل الكاذب في الأغنام: المسببات البكتيرية وحساسيتها لبعض المضادات الميكروبية معملياً

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معهد بحوث صحة الحيوان بأسبوط

يعتبر مرض السل الكاذب من أهم المشكلات التي تؤثر سلبياً من الناحية الاقتصادية والإنتاجية على الأغنام. وقد أجريت هذه الدراسة على عدد ٧٦٢ رأساً من الأغنام التي تربي في قطعان أهلية من مختلف الأعمار، وفي مناطق مختلفة بمحافظة أسبوط [٦١٦ رأساً من الإناث و ١٤٦ رأساً من الذكور]. أظهرت نتائج الفحص الإكلينيكي أن ٢٢,٧٪ من الأغنام كانت مصابة بالأعراض الظاهرية لمرض السل الكاذب [تضخم في الغدة الليمفاوية]، بينما كانت ٢٠,١٪ فقط ايجابية للعزل البكتيري. ووجد اختلافات معنوية عالية بين الإناث (٢٥,٣٪) والذكور (١١,٦٪) المصابة. كما وجد اختلافات معنوية عالية في معدل انتشار المرض بين الأعمار المختلفة وأن أعلى معدل لانتشار المرض كان في الأغنام التي يتراوح أعمارها من ١-٢ سنة (٣٧,٣٤٪) يليها في الأغنام التي عمرها ٢-٣ سنوات (٢٥,٧٪)، بينما كان أقل معدل لانتشار المرض في الأغنام التي عمرها أقل من سنة واحدة (٨,٣٣٪). مثلت الغدة الليمفاوية النكافية أعلى نسبة إصابة (١١,٨١٪) تلتها الغدة الليمفاوية قبل عظمة اللوح (٧,٨٧٪)، على عكس الغدة الليمفاوية أمام الفخذ كانت أقل في نسبة الإصابة (٠,٣٩٪).

أظهرت النتائج أن الغدة الليمفاوية السطحية في الجزء الأمامي من الجسم أعلى في نسبة الإصابة عن الغدة الليمفاوية السطحية في الجزء الخلفي من الجسم. بينت نتائج الفحص البكتيريولوجي أن ميكروب كوريني السل الكاذب هو المسبب الرئيسي للمرض حيث أمكن عزله من الحالات التي ظهرت عليها الأعراض الإكلينيكية للمرض أما بصورة منفردة من ١٤٢ حالة (٨٢,١٪) أو بصورة مختلطة مع الميكروب المكور العنقودي الذهبي من ٧ حالات (٤٪)، كما أمكن عزل الميكروب المكور العنقودي الذهبي في صورة منفردة من ٤ حالات (٢,٣٪). كانت ٩٧,٣٪ من عترات ميكروب كوريني السل الكاذب المعزولة سلبية لاختبار اختزال النترات.

وبإجراء اختبارات الحقن في حيوانات التجارب أظهرت جميع عترات ميكروب كوريني السل الكاذب المعزولة ضراوة شديدة عند حقن خنازير غينيا. أظهرت نتائج اختبار الحساسية لبعض عترات ميكروب كوريني السل الكاذب المعزولة للمضادات الميكروبية معملياً أن كلاً من أنروفلوكساسين وسبيروفلوكساسين لهما تأثير مثبت قوي على جميع عترات ميكروب كوريني السل الكاذب المختبرة، بينما أظهرت العترات مقاومة عالية لكل من لينكوميسين، ستربتوماسين، أموكسيسيلين والبنسلين. وتبين من الدراسة أن ميكروب كوريني السل الكاذب هو المسبب الرئيسي لمرض السل الكاذب في الأغنام، الغدة الليمفاوية السطحية في الجزء الأمامي من الجسم أعلى في نسبة الإصابة عن الغدة الليمفاوية السطحية في الجزء الخلفي من الجسم وأن كلاً من أنروفلوكساسين وسبيروفلوكساسين من أفضل المضادات الميكروبية معملياً.