Dept. of Bacteriology, Mycology and Immunology,
Fac. Vet. Med., Cairo Univ., Beni-Seuf Branch.

SEROLOGICAL AND IMMUNOLOGICAL STUDIES ON *CORYNEBACTERIUM PSEUDOTUBERCULOSIS*

(With 2 Tables)

By

F.R. EL-SEEDY; S.A. SELIM*; I.A. RADWAN and HALA S. HASSAN

* Dept. of Microbiology, Fac. Vet. Med., Cairo Univ.,

(Received at 26/6/2005)

دراسات سيرولوجية ومناعية عن عصيات السل الكاذب

فوزي رياض الصعيدي ، صلاح الدين عبد الكريم سليم ، إسماعيل عبد الحفيظ رضوان ، هالة سيد حسن

تم التخطيط لهذا العمل لدراسة العلاقة بين المناعة السائلة والعدوى الاكلينيكية وتحت الاكلينيكية للاصابة بالتهاب الغدد الليمفاوية التكرزى وامكانية استخدام اختبار الاليزا فى تشخيص هذا المرض وأيضا لتقييم دور أربعة لقاحات مختلفة فى تحفيز القوة القاتلة للخلايا الأكولة. تم جمع عشرون عينة مصل من أغنام تبدو عليها إصابات اكلينيكية مشابهة للمرض وعشرون عينة من أغنام سليمة ظاهريا متواجدة فى ذات المكان وكذلك عشرون عينة مصل سالبة تم جميعها من أغنام عمر ستة أشهر تعيش فى منطقة خالية من المرض لحساب القيم النهائية للانتيجين المستخدم فى اختبار الاليزا. بالفحص البكتريولوجى لعشرين مسحة تم تجميعها من غدد ليمفاوية متكرزة تم عزل ثلاث عترات من ميكروب السل الكاذب. بالفحص السيرولوجى لعدد أربعين عينة مصل ممثلة للمجموعتين تحت الدراسة باستخدام اختبار الاليزا وباستخدام نوعان مختلفان من الأنتيجينات وهما الفوسفوليبيزا والأنتيجين الجسمى وجد أن هناك ١٨ عينة إيجابية من المجموعة الأولى عند استخدام أنتيجين الفوسفوليبيزو ، و١١ عينة إيجابية عند استخدام الأنتيجين الجسمى. أما بالنسبة للمجموعة الثانية، فكان عدد الحالات الايجابية ١٨ عند استخدام أنتيجين الفوسفوليبيزو، و٨ عينات إيجابيةعند استخدام الأنتيجين الجسمى. تم دراسة الأنتيجينات المختلفة (اللقاحات) على نشاط الخلايا الأكولة عن طريق حقن أربع مجموعات من فئران البالب سى. كل مجموعة تتكون من خمسة فئران وذلك باستخدام أربعة أنتيجينات هى التوكسويد، البكتريا الميتة. البكتريا الحية، ولقاح مشترك (التوكسويد + البكتريا الميتة). تم تجميع الخلايا الأكولة من تجويف البطن من مجموعات الفئران المحصنة ثم نقلها لفئران طبيعية (فئران مستقبلة) والتى تم استنفارها بميكروب السل الكاذب الحى (٢ x ١٠) وذلك بعد تجميع الخلايا الأكولة من كل مجموعة لتحديد قدرتها القتلية للميكروب. وأظهرت النتائج أن اعلى قدرة قتلية كانت للخلايا التى تم جمعها من المجموعة المحصنة بالتوكسويد يليها اللقاح المشترك ثم المجموعة المحصنة بالبكتيريا، وأخيرا المجموعة المحصنة بالبكتريا الميتة.

SUMMARY

This work was planned to investigate the correlation between humoral immune response, clinical and subclinical infection and the possibility of using ELISA test for diagnosis of caseous lymphadenitis (CLA) also, to evaluate the role of four types of vaccines in killing activity of macrophages. Twenty serum samples were collected from sheep showing clinical lesions suspected to be caseous lymphadenitis, also, twenty serum samples were collected from apparently normal incontact sheep and twenty control sera were collected from 6 month-old sheep living in free area. The serum samples of control group were used for calculation of the cut off values for ELISA antigens. Out of the twenty bacteriologically examined swabs collected from caseated lymph nodes, three isolates of *C. pseudotuberculosis* were recovered. Out of serologically examined 20 serum samples collected from sheep with characteristic CLA lesions, 18 serum samples were found positive by using Phospholipase D (PLD) antigen and 11samples were found positive when the somatic antigen was used as a coating antigen. On the other hand, incontact sheep sera showing positive results in 18 out of 20 sera when examined against PLD and 8 were positive when examined against somatic antigen. The killing activity of peritoneal macrophages collected from all groups of vaccinated to recipient mice received living *C. pseudotuberculosis* was estimated and the obtained results were as follows: Group of recipient mice inoculated with macrophages sensitized with combined vaccine (phospholipase D+ Bacterin) yielded 7 bacterial colonies compared with 23 colonies in control mice received living *C. pseudotuberculosis* only. Group of mice inoculated with macrophage sensitized with toxoid (PLD) yielded 3 bacterial colonies compared with 58 colonies in control mice. The third group which was sensitized with Bacterin yielded 46 colonies compared with 58 bacterial colonies in control mice. The fourth group which comprise living *C. pseudotuberculosis* sensitized macrophages recorded 11 bacterial colonies in recipient mice and 55 bacterial colonies in control mice.

Key words: *Corynebacterium pseudotuberculosis, serology, immunology.*

INTRODUCTION

Caseous lymphadenitis caused by *Corynebacterium pseudotuberculosis* (*C. pseudotuberculosis*), is a chronic disease of sheep, goats and other small ruminants. Clinical signs and lesions may

not be observed until several months after initial infection and the disease may be manifested as abscesses located internally in organs and lymph nodes (Stoops *et al.*,1984). On the other hand, the early diagnosis in a flock may be difficult, as subclinical infection occur (Brown *et al.*, 1987). However, serological studies have proved to be suitable for the detection of the infected animals. (Schreuder *et al.*, 1994) and Prescott *et al.*, (2002).

The immunological mechanism of protection against infection with *C. pseudotuberculosis* in sheep and goats still a matter of controversy. Depending on data obtained from immunological analysis of naturally or artificially infected sheep and goats with caseous lymphadenitis (CLA), investigations are differentiated into three categories. Those hypothesized that immunity against CLA depends mainly on humoral immune response with specific antibodies against *C. pseudotuberculosis* (Hodgson *et al.*, 1994) but no one detect the protective antigen of the causative agent either in the secretory part which includes exotoxin (s) or in the cell wall. Meanwhile, many investigators reported that protection against CLA is a matter of cell mediated immune response and endowed by increase of killing activity of macrophages against phagocytosed Corynebacteria and hypothesized this cell mediated views depending upon the fact that *C. pseudotuberculosis* is a facultative intracellular microorganism (Tashjian and Compbell, 1983 and Johnson *et al.*, 1993). The third division of investigators support the view of development of both types of immune response which are necessary for protection against infection with *C. pseudotuberculosis* (Cameron *et al.*, 1998).

The present study was conducted to throw a light on:

1. The correlation between humoral immune response, clinical and subclinical infection and the possibility of using ELISA test for diagnosis of CLA.

2. Evaluation of the role of different types of vaccines on killing power of macrophages.

MATERIALS and METHODS

Samples:

A. Serum samples:

Sixty serum samples were collected from sheep flocks with average age ranged from 6 months up to five years-old of both sexes. These samples were as follow:

1- Twenty serum samples were collected from sheep showing clinical lesions suspected to be caseous lymphadenitis. The lesions were in the form of lymph node abscesses specially the prescapular, prefemural, and submandibular lymph nodes.
2- Twenty serum samples were collected from apparently healthy in contact sheep.
3- Twenty serum samples were collected from six months-old sheep living in free area. These sera act as a control negative sera.

B. Bacteriological samples:

Samples were collected from all caseated lymph nodes for bacteriological examination of sheep suffered from lesions suspected to be caseous lymphadenitis.

Media used for isolation of the microorganism:

Sheep blood agar and tryptone soy agar, brain heart infusion agar and FNR medium were used for recovery of *Corynebacterium pseudotuberculosis*.

Identification of the isolates:

Isolates were identified according to Songer *et al.*, (1988) and Koneman *et al.*, (1992).

Modified CAMP test:

Synergistic haemolysis was applied after Songer *et al.*, (1990) using Luria-Bektani (LB) agar containing 5% sheep blood and 10% *Rhodococcus equi* (*R. equi*) filtrate.

Serological diagnosis of caseous lymphadenitis by Enzyme Linked Immunosorbent assay (ELISA):

This test was applied on 60 collected serum samples using *C. pseudotuberculosis* somatic antigen prepared according to Ellis *et al.*, (1990) and phospolipase D antigen (PLD antigen) (Biotechnology Center, Fac. Vet Med., Cairo Univ.)

Optical density measurement:

A serum dilution was considered positive if it yielded a mean OD of each group equal to/or greater than the cut off value (Dimitri and Mikhail, 1996).

Cut off value was estimated as double or more fold of the mean OD of negative serum. (Bassiri *et al.*, 1993)

Effects of different vaccines on macrophage activity:

Preparation of vaccines:

1- Living *C. pseudotuberculosis*:

One single colony of completely identified local isolate of *C. pseudotuberculosis* was inoculated into brain heart infusion broth

containing 0.1% Tween 80, incubated for 48hr at 37°c in the shaking incubator. The number of the bacterial cells was counted by standard curve and the culture was centrifuged at 3000rpm for 10 min at 4°c. The supernatant was discarded and the pellet was resuspended in physiological saline. The number of resuspended cells was adjusted to contain $1x10^4$CFU/ml.

2-Bacterin:

The dried bacterial pellets were weighed and resuspended in 1% formalin saline solution at a concentration of 10mg washed bacterial cells/ml. Tween80 was added till a final concentration of 3.4%. The vaccine was checked for sterility after 12hr incubation at room temperature.

3- Culture filtrate vaccine (Toxoid):

Kindly selected colony was inoculated into 250ml brain heart infusion broth containing 0.1% Ttween80 and incubated in shaking incubator for 72hr.The culture was centrifuged at 6000rpm for 15min, filter sterilized and the total protein in 1ml filtrate was calculated by Lawrie, (1991). One ml of filtrate was concentrated to 1/10 of its volume. The concentrated toxin was detected by performing of gel electrophoresis. The amount of toxin (PLD) was calculated and inactivated by formalin to 1.0% concentration.

4-Combined vaccine (PLD+Bacterin):

Each ml containing 50µg PLD and 10mg bacterin in water-in-oil-adjuvant.

Evaluation of the prepared vaccines:

Four groups of 6 weeks old female Balb/C mice each group of 5 mice

The first group:

Inoculated s/c with 0.2ml of the combined vaccine then boostered after 4weeks.

The second group:

Inoculated s/c with 5µg toxoid/mouse and boostered after 4weeks.

The third group:

Subcutaneously inoculated with 0.2ml of the prepared bacterin/mouse and boostered after 4weeks.

The fourth group:

Each mouse received 0.2ml of living *C. pseudotuberculosis* saline suspension at a concentration of $1x10^4$CFU/ml.

Macrophage transfer:

Peritoneal macrophages were collected from all groups of vaccinated mice according to Cameron and Engelbrecht (1971).

Four groups of recipient mice (3 mice for each vaccine) were inoculated intraperitoneally with 0.5ml dose of $3x10^6$CFU/ml macrophages.

All recipient mice were challenged 18hr later with 0.5ml of $2x10^6$CFU/ml living *C. pseudotuberculosis* intraperitoneally. Three control mice were also challenged with the same dose of the living organism.

Enumeration of live bacteria in the peritoneal macrophages:

One hour following challenge, peritoneal macrophages were collected, ten fold serial dilutions was made, duplicate plates were prepared by spreading 0.1ml of the 10^{-2} and 10^{-3} dilutions on brain heart infusion agar. The colonies were counted after 48h incubation at 37°c.

RESULTS

Incidence of *C. pseudotuberculosis* recovered from caseated lymph nodes:

Out of the bacteriologically examined 20 lymph node samples collected from sheep with lesions suspected to be CLA, 3 isolates were proved to be *C. pseudotuberculosis* when characterized biochemically and with CAMP test.

Results of ELISA test on sera collected from examined groups of sheep:

Twenty serum samples collected from 20 apparently healthy sheep living in free area and less than 6 months old were divided into two subgroups for examination against two coating antigens, namely, the somatic and the phospholipase D antigens.

These negative control sera were used for calculation of the cut off values. The results indicated that, the cut off value was 0.264 for somatic and 0.268 for PLD antigen.

Out of the serologically examined 20 serum samples collected from sheep with characteristic CLA lesions, 18 serum samples were found positive by using PLD antigens, meanwhile, 11 serum samples were found positive when the somatic antigen was used as a coating antigen (Table 1).

On the other hand, 20 serum samples collected from in contact sheep with no clinical lesions of CLA revealed that 18 samples were found positive with PLD antigen and 8 samples reacted positively against somatic antigen.

Results of the effects of different types of vaccines under study on macrophages:

The killing activity of peritoneal macrophages collected from all groups of vaccinated to recipient mice received living *C. pseudotuberculosis* was estimated and the following results were obtained:

Group of recipient mice inoculated with macrophage sensitized with combined vaccine (PLD+ Bacterin) yielded 7 bacterial colonies compared with 23 colonies in control mice received living *C. pseudotuberculosis* only.

Group of recipient mice inoculated with macrophage sensitized with toxoid (PLD) yielded 3 bacterial colonies compared with 58 colonies in control mice.

The third group of recipient mice (Bacterin sensitized macrophages) yielded 46 colonies while control mice were harboured with 58 bacterial colonies.

The fourth group (Living *C. pseudotuberculosis* sensitized macrophages) recorded 11 bacterial colonies in recipient mice and 55 bacterial colonies in control mice. (Table 2)

Table 1: Collective ELISA and bacteriological results of samples obtained from sheep with lesions suspected to be CLA.

Sample No.	OD values of somatic antigen	Interpretation	OD values of PLD antigen	Interpretation	Culture results
1	0.338	+	0.680	+	-
2	0.213	-	0.294	+	-
3	0.281	+	0.456	+	+
4	0.261	-	0.237	-	-
5	0.733	+	0.732	+	-
6	0.921	+	0.902	+	-
7	0.417	+	0.479	+	-
8	0.563	+	0.811	+	-
9	0.421	+	0.738	+	-
10	0.226	-	0.380	+	-
11	0.062	-	0.479	+	-
12	0.172	-	0.591	+	-
13	0.489	+	0.410	+	-
14	0.115	-	0.365	+	-
15	0.268	+	0.507	+	-
16	0.167	-	0.490	+	-
17	0.327	+	0.577	+	-
18	0.287	+	0.633	+	+
19	0.177	-	0.381	+	+
20	0.083	-	0.226	-	-

Table (2): Effect of different vaccines on the killing activity of macrophages.

Type of vaccine	Vaccinated mice		Recipient mice		Control mice		No. of Lysed macrophages		No. of colonies /100µl	
	Total leukocytic count/ml	Total macrophage count /ml	Total leukocytic count/ml	Total macrophage count /ml	Total leukocytic count/ml	Total macrophage count /ml	Recipient	Control	Recipient	Control
Combined vaccine	1.9×10^7	6×10^6	8.4×10^6	3.7×10^5	5.8×10^6	1.6×10^6	116,000	116,000	7	23
Toxoid (PLD)	2.9×10^7	8×10^6	2.8×10^7	10.7×10^6	4.8×10^6	1.16×10^6	116,000	116,000	3	58
Bacterin	2.1×10^7	6.1×10^6	3.2×10^7	99×10^5	2.7×10^7	79×10^5	972,000	972,000	46	55
Living vaccine	2.3×10^7	6.4×10^6	3.9×10^7	86×10^5	2.7×10^7	97×10^5	972,000	972,000	11	55

DISCUSSION

Caseous lymphadenitis caused by *C. pseudotuberculosis* is a chronic disease of sheep,goats and other small ruminants which characterized by delayed clinical signs and lesions.

Out of 20 examined bacteriological swabs collected from caseated lymph nodes, 3 typical isolates (15%) of *C. pseudotuberculosis* were recovered. Sahar Abdel-Lattif (1998) recovered 11 isolates of *C. pseudotuberculosis* when examined 126 sheep lymph nodes with typical CLA lesions.

In this study, an ELISA for detection of antibodies directed against the somatic and PLD antigens was developed. For interpretation of ELISA results, 20 serum samples collected from sheep showing characteristic CLA lesions, 20 samples collected from clinically CLA negative in contact sheep and 20 control negative serum samples were used.

The results indicated that 18 and 11 serum samples out of the 20 samples collected from clinically diseased sheep were found ELISA positive using PLD and somatic antigens respectively. On the other hand, 18 and 8 serum samples collected from incontact sheep were found positive using PLD and somatic antigens respectively.

Using cell wall and PLD ELISA for serological studies on goats suffering from caseous lymphadenitis was recommended by Braithwaite *et al.*, (1993) and Sting *et al.*, (1997).

Several factors may contribute to the apparent lack of specificity of *C. pseudotuberculosis* ELISA when applied for diagnosis of naturally acquired infections. First, there is inter-host variability including the age and immune status of the infected animal, the route and extent of exposure to the pathogen and the interval between exposure and serologic analysis. Second, all current assay use complex, poorly defined antigens (Maki *et al.*, 1985). Third, unidentified strain differences may exist between isolates of *C. pseudotuberculosis* causing disease in sheep and the standard strain that was used as a source of antigens (Takai *et al.*, 1987).

Vaccination of sheep against CLA stills a matter of controversy. Some authers believed in the antibacterial immunity and recommended bacterins or cell wall antigens or even living Corynebacterial cells as vaccines. Other authers confirmed that immunity against Corynebacteria is primary antitoxic and preferred vaccination with toxoid. The third

division of authers believes in the importance of both antibacterial and antitoxic immunity and recommended the use of combined vaccines.

A substantial portion of this study was concerned with attempts to elucidate the role of macrophages as one of the important components of cellular immunity against infection with *C. pseudotuberculosis* and if any of the different types of vaccines recommended can stimulate and increase the activity of macrophages for killing phagocytosed *C. pseudotuberculosis.*

Four groups of Balb/C mice were vaccinated with four types of recommended vaccines. The killing assay of macrophages collected from each group was performed by collection of macrophages from each group and transferred to the intraperitoneal cavity of a recipient mice which was challenged by a fixed number of living *C.pseudotuberculosis.*

Killing assay was performed by collection of peritoneal macrophages from normal mice challenged with the live organism and equal number of macrophages collected from each group were lysed and cultivated to count the number of liberated living Corynebacteria.

The least number of living Corynebacteria was obtained from macrophages collected from mice group vaccinated with toxoid. The count was CFU and it was 3 and 58 CFU in case of recipient group and control group respectively. This result coincide with the data of other investigators that recommended toxoid as the most effective vaccine against CLA depending upon the fact that the exotoxin of *C. pseudotuberculosis* (Phospholipase D) function in vivo as a permeability factor, allowing for local spread of the organisms and increasing chance that the bacteria may be carried to more distant site and antitoxin antibodies in the initial bacterial multiplication period may limit local spread and general dissemination of the organism (Brown *et al., 1986*). Also, Hodgson *et al* (1999) revealed that formalin-inactivated toxin offered 95% protection to challenge sheep with live *C. pseudotuberculosis*. The adverse effect of toxins on the activity of macrophages was studied by Sergguei *et al.* (2002) who noticed the inhibition of phagocytic activity of macrophages treated with sublytic concentration of lethal anthrax toxin. Also, the adverse effect of toxins on activity of macrophages confirmed by Sampaio *et al.* (2003) who reported that crotoxin when s/c injected to rats or added to the medium of peritoneum cell incubation, inhibited macrophages function in a similar manner to that observed on crude snake venom.

Macrophages collected from mice vaccinated with whole inactivated component cell vaccine (Bacterin) are far weak in killing

activity of *C. pseudotuberculosis* if compared with those collected from toxoid vaccinated mice. These results agree with the low validity observed by many authors who vaccinated sheep or laboratory animals with bacterin. The efficacy of bacterin have been reported to be partially successful (Collinus and Campbell, 1982; Brodgen *et al.*,1990 and 1996).

As regards to the protective effect of *C. pseudotuberculosis* vaccine, the killing activity of collected immune macrophages was performed and it was far less than the other forms of vaccines except the killed vaccines.

This weak efficacy in killing phagocytosed bacteria can be attributed to the adverse effect of exotoxin produced by the invasive *C. pseudotuberculosis* inside the tissues of the host on phagocytic cells, in addition to the toxic effect of phagocytosed Corynebacteria on the macrophages itself and that may lead to death of macrophages within 24 hours. This low efficacy of macrophages collected from inoculated mice with living *C. pseudotuberculosis* can be noticed in the results of some authors that used living *C. pseudotuberculosis* in vaccination of sheep and goats (Hodgson *et al.*, 1992; Johnson *et al.*, 1993 and Cameron *et al.*, 1998). The effect of combined vaccine was investigated and the number of CFU was higher if compared with that of macrophages collected from mice inoculated by toxoid alone. Holstad *et al.*, (1989) vaccinated goats with a combined vaccine and reported that the used vaccine was not sufficiently effective to be recommended as the only protective measure against CLA in goats. At the same time Point-kowski *et al.*, (1998) evaluated the commercially available bacterin-toxoid vaccine in sheep and concluded a substantial decrease in the prevalence and number of abscesses that formed secondary to *C. pseudotuberculosis* infection.

REFERENCES

Bassiri, M.; Ahmed, S.; Giavendoni, L.; John-Satiki, J.T.; Mebus, J. and Yilma, T. (1993): Immunological response to baculovirus expressed f and H. J. Virology, 67: 1255-1261.

Braithwaite, C.E.; Smith, E.E.; Songer, J.G. and Reine, A.H. (1993): Characterization of detergent-soluble proteins of *C. pseudotuberculosis*. Vet. Microbiol., 38 (1-2): 59-70.

Brodgen, K.A.; Cutlip, R.C.; Lemkuhl, H.D. and Sacks, J. (1990): Effect of muramyl dipeptide on immunogenicity of *C. pseudotuberculosis* whole cell vaccines in mice and lambs. Am. J. Vet. Res., 51: 200-202.

Brodgen, K.A.; Glenn, J.S.; East, N. and Audibert, F. (1996): C. pseudotuberculosis bacterin with muramyl dipeptide induces antibody titers, increases the time of onset, and decreases naturally occurring external abscesses in sheep and goats. Small Rum. Res., 119(2): 1161-168.

Brown, C.C.; Olander, H.J.; Biberstein, E.L. and Morse, S.M. (1986): Use of a toxoid intradermal challenge exposure to *C. pseudotuberculosis*. Am. J. Vet. Res., 47: 1116-1119.

Brown, C.C.; Olander, H.J. and Alues, S.F. (1987): Synergestic hemolysis-inhibition titres associated with caseous lymphadenitis in slaughter houses. Survey of goats and sheep in Northeastern Brazil. Can.J.Vet. Res.,51(1): 46-49.

Cameron, C.M. and Engelbrecht Maria, M. (1971): Mechanism of immunity to *C. pseudotuberculosis* in mice using inactivated vaccine. Onderstepoort J. Vet. Res., 38(2): 73-82.

Cameron, P.S.; Sarah, J.D.; Mlary, T.; Aaolanta, K.; Adrian, L.M.; Hodgson and Richard, A.S. (1998): Vaccine potential of attenuated mutants of *C. pseudotuberculosis* in sheep. Infect. And Immunol., 66 (2): 474-479.

Collinus, F.M.; Campbell, S.G. (1982): Immunity to intracellular bacteria Vet. Immunol. Immunopathol., 3: 5-66.

Dimitri, R.A. and Mikhail, D.G. (1996): Specific skin reactivity and ELISA for the diagnosis of bovine tuberculosis using 30000 Da and PPD antigens in Guinea pigs. J. Egypt Vet. Med. Assoc., 56(4): 446-531.

Ellis, J.A.; Hawk, B.S.; Holler, L.D. and Mills, K.W. (1990): Differential antibody responses to *C. pseudotuberculosis* in sheep with naturally acquired caseous lymphadenitis. JACMA, 196 (10): 1609-1613.

Hodgson, A.L.M.; Carter, K.; Tachedjian, M.; Krywult, J.; Corner, L.A.; McColl, M. and Cameron, A. (1999): Efficacy of an ovine caseous lymphadenitis vaccine formulated using a genetically inactive form of the *C. pseudotuberculosis* phospholipase D. Vaccine, 17 (7/8): 802-808.

Hodgson, A.L.M.; Krywlt, J.; Corner, L.A.; Prothel, J.S. and Raadford, A.J. (1992): Rational attenuation of *C. pseudotuberculosis* : potential cheesy gland vaccine and live delivery vehicle. Infect. And Immunol.,60(7): 2900-2905.

Hodgson, A.L.M.; Tachedjian, M.; Corner, L.A. and Radford, A.J. (1994): Protection of sheep against caseous lymphadenitis by use of a single oral dose of live recombinant *C. pseudotuberculosis.* Infect. And Immunol., 62 (12): 5275-5280.

Holstad, G.; Teige, J.; JR. and Jarsen, H.J. (1989): C. pseudotuberculosis infection in goats. VIII. The effect of vaccination against experimental infection. Veterinaria Scandianavica, 30(3): 275-283.

Johnson, E.H.; Santa Rosa, J. and Kass, P.H. (1993): Immunizing effects of *C. pseudotuberculosis* in goats. Small ruminant research, 12 (3): 349-356.

Koneman, K.W.; Allen, S.D.; Dowell, V.R. and Sommers, H.M. (1992): Colour Atlas and Textbook of Diagnostic Microbiology. 2nd Ed. J. B. Lippicott Co., London.

Lawrie, R.A. (1991): Meat Science 5th ed. Pergamon Press, England.

Maki, L.R.; Shen, S.H.; Nergstrom, R.C. and Wstetzbanch, L.D. (1985): Diagnosis of *C. pseudutuberculosis* infections in sheep. Am. J. Vet. Res., (8): 2294-298.

Point-Kowski, D.V.M. and Douglas, W. Shiuvers (1998): Evaluation of a commercially available vaccine against *C. pseudotuberculosis* for use in sheep. JAVMA Vol. 212 No. 11: 1765-1768.

Prescott, J.F.; Menzies, P.I. and Hwang, Y.T. (2002): Interferon-gamma assay for diagnosis of *C. pseudotuberculosis* infection in adult sheep from a research flock. Vet. Microbiol., 88 (3): 287-297.

Sahar, G. Abdel-Lattif (1998): Selective medium and suitable antigen for improving diagnosis of infection with Corynebacterium pseudotuberculosis. M.V.Sc. Thesis, Fac. Vet. Med., Cairo Univ.

Sampaio, S.C.; Brigaatte, P.; Sousa-e-Silva, M.C.C.; dos-santos, E.C.; Rangel-Santos, A.C. Curi, R.; Cury, Y. (2003): Contribution of crotoxin for the inhibitory effect of crotalus durissus terrificus snake venom on macrophage function. Toxicon, 41: 889-907.

Schreuder, B.E.C.; Laak, E.A. and Dercksen, D.P. (1994): Eradication of caseous lymphadenitis in sheep with the help of a newly developed ELISA techniquee. Vet. Rec., 135 (8): 174-176.

Sergguei, G.; Dopov, Rafacel Vittatingly, Jessica Bernardi, Edth Grene; Jennifer Cardwell; Aiguo, W.U.; Darya Alibek; Charles Bailey and Ken Alibek (2002): Lethal toxin of Bacillus anthracis causes apoptosis. Biochem. and Biophysic. Res. Com., 2293: 349-355.

Songer, J.G.; Beckenbach, K.; Marshall, M.M.; Olson, G.B. and Kelleey, L.(1988): Biochemical and genetic characterization of *C. pseudutuberculosis*. Am. J. Vet. Res., 49(2): 223-226.

Songer, J.G.; Libby, S.J.; Iandola, J.J. and Cuevas, W.A. (1990): Cloning and expression of the phospholipase from *C. pseudotuberculosis* in *Escherichia coli* Infect. Immunol., 58(1): 131-136.

Sting, R.; Steng, G. and Spengler, D. (1997): Serological studies on *C. pseudotuberculosis* infections in goats using enzyme-linked immunosorbent assay. J. Vet. Med., 45: 209-216.

Stoops, S.G.; Renshaw, H.W. and Thisted, J.P. (1984): Ovine caseous lymphadenitis: disease prevalence, lesion distribution and thoracic manifestations in a population of mature culled sheep from Western United States. Am. J. Vet. Res., 45(3): 557-561.

Takai, S.; Kawazu, S. and Tsubaki, S. (1987): humoral response of foals to experimental infection with Rhodococcus equi. Vet. Microbial., 14: 321-327.

Tashjian, J.J. and Campbell, S.G. (1983): Interaction between caprine macrophages and *C. pseudotuberculosis* in an electron microscopic study. Am. J. Vet. Res., 44: 690-693.