

CASEOUS LYMPHADENITIS IN IRAQI SHEEP AND ASSESSMENT OF VACCINATION WITH COMMERCIAL VACCINE

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Keywords; lambs, *C. pseudotuberculosis*, challenge dose.

ABSTRACT

Experimental study included 15 lambs which were divided into 3 groups; each group consisted of 5 lambs. A commercial Case- Bac vaccine was used to vaccinate group II, while others groups served as positive and negative controls. All lambs in group II were received two doses of vaccine at four weeks interval. The lambs in groups II and III were challenged S/C with 9×10^8 CFU virulent isolated strain of *C. pseudotuberculosis*.

Humoral and cell mediated immune response were detected during the period of experiments, the temperature, pulse rate and respiration rate were determined weekly.

The protection in vaccinated group was 100% against challenge dose, while in the group III one lamb died on fifth day post challenge. Pus was appeared in the rest of lambs at the site of injection.

INTRODUCTION

Caseous lymphadenitis (CLA) is a chronic infectious disease of small ruminant (sheep and goats) caused by the bacterium *Corynebacterium pseudotuberculosis* was formerly known as *Corynebacterium ovis* (1).

The disease found in the major sheep and goat production all over the worlds which was cause significant economic losses due to culling of affected animals, decrease in reproductive efficiency in meat, wool and milk production, carcass and skin condemnation due to abscessation (2).

The best strategy for control and prevention of CLA disease is immunization especially in countries with high prevalence of infection (3). To make the control of CLA successful, the identification of infected animals are necessary. Bacteriological culture is mandatory to done to exclude other bacterial pathogens capable to produce suppurative lesions (4).

This study was conducted to know the efficacy of commercial vaccine (Case-Bac Vaccine) by immunization trials against the caseous lymphadenitis disease in sheep and study cellular and humoral immunity.

MATERIALS AND METHODS

Experimental Animals:

Fifteen male lambs Awassi breed their ages ranged between 5-7 months old were used in this study. The animals farmed in college of veterinary medicine university of Baghdad. All lambs were treated by Ivermectin 200 µg per kg of body weight subcutaneously at the rate of 1 ml per 50 kg as anthelmintic treatment for internal and external parasite before starting the experiment and remained one month for adaptation. Lambs divided into 3 equal groups (I, II, and III) each group consisted of 5 lambs.

All lambs were clinically healthy and immunological response to infection was negative as determined by measurement of serum anti PLD (phospholipase D) IgG by Enzyme Linked Immunosorbent assay (ELISA) test.

CASE-BAC vaccine:

A commercial vaccine prepared by Colorado Serum Company (U.S.A.), detoxified and purified the whole culture of *Corynebacterium pseudotuberculosis* contains thimerosal as preservative used in healthy sheep as a killed bacterin-toxoid

vaccine. The vaccine injected subcutaneously in a dose of 2 ml in axillary space and repeated in four weeks in opposite axillary space.

ELITEST CLA:

An Enzyme Immuno-Assay for the detection of IgG antibodies specific for the *Corynebacterium pseudotuberculosis* in sheep sera. The (ELISA) utilized a recombinant form of phospholipase D (PLD) from *C. pseudotuberculosis* to detected anti-PLD IgG antibodies in sera of sheep and goats infected with caseous lymphadenitis. The commercial kit ELITEST CLA product of Hyphen BioMed, France was used for detection of IgG antibodies specific for the causative agent of caseous lymphadenitis (CLA) in sheep and goat sera. This kit was performed according to the manufactures instruction, recombinant phospholipase D (rPLD) antigen used for determined antibodies in the sera of animals.

Cell Mediated Immune Response:

Cellular immune responses of the vaccinated and control groups were evaluated by Delayed Type of hypersensitivity (DTH) skin test.

It was done two weeks after booster dose of vaccination. Delayed Type of hypersensitivity was assessed by the skin thickness that was measured before and after the inoculation of *C. pseudotuberculosis* antigen (5)

The prepared soluble Ag was given intra dermally in the lower flank region. The skin measurement was done by using metal Vernier caliper micrometer to estimate double skin fold thickness before and after the I/D injection with 0.1 ml Ag contained 0.5 mg protein which was determined by means of (6 and 7).

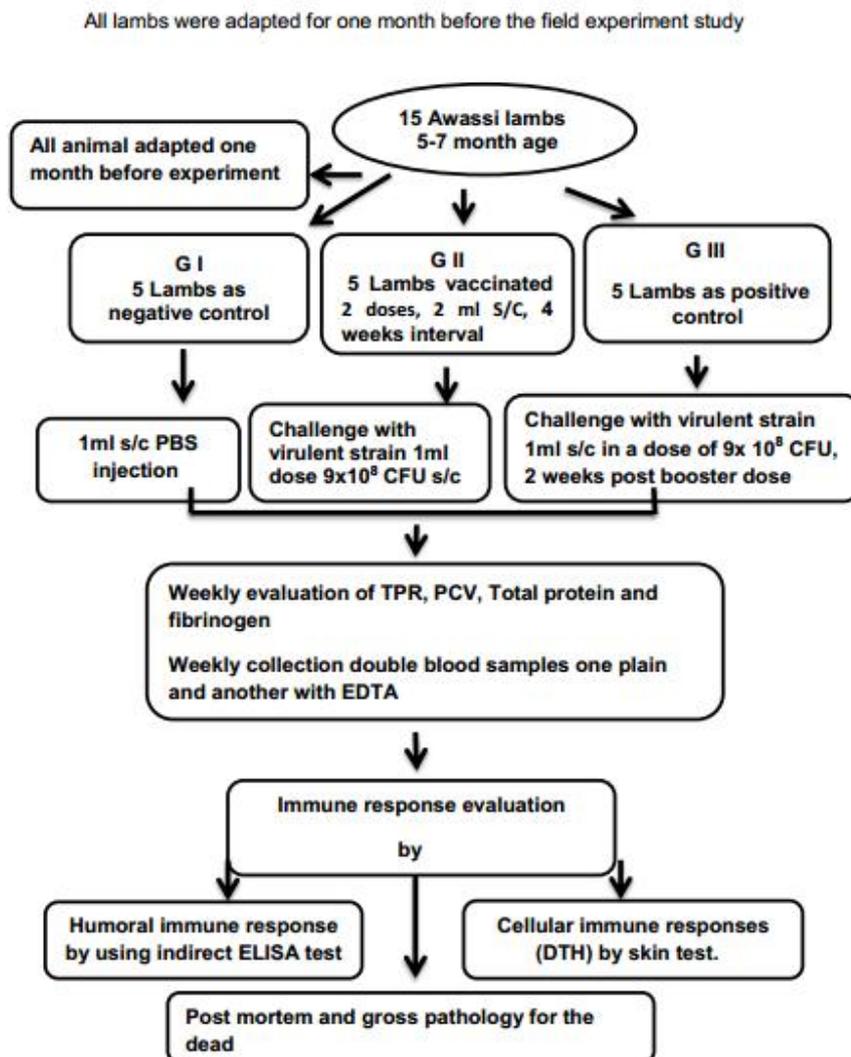
Preparation of *C. pseudotuberculosis* inoculum:

The bacterium was cultured on blood agar plate and then growth was collected by using sterile cotton swabs. Bacteria were suspended in phosphate buffered saline (PBS) in sterile test tubes by rolling swab gently against the tube sides. The bacterial suspension

was adjusted to a final concentration of (9×10^8) CFU per ml for challenge dose. MacFarland tubes were used to estimate colony forming unit (CFU) as mentioned by (8).

One ml inoculum was given subcutaneously in the flank region about 10 cm above the pre femoral lymph node of each lamb.

Experimental design:



Statistical analysis:

All data were analyzed statistically using one way ANOVA test by Microsoft program (SPSS). The level of statistical significance was set at ($P < 0.05$) as described by (9). The chi-square test was achieved to compare between percentage of the study.

RESULTS

The clinical examination of group II (G II) moderate increase in rectal temperature, pulse, and respiration (40.24 ± 0.45 °C), (98 ± 3.16 / minute), and (41.6 ± 3.85 / minute) respectively were recorded after vaccination as showed in Figures (1, 2, and 3). All lambs returned to their normal activities within 3-4 days post vaccination. Slight localize swelling was noticed at the sight injection and disappeared within one week.

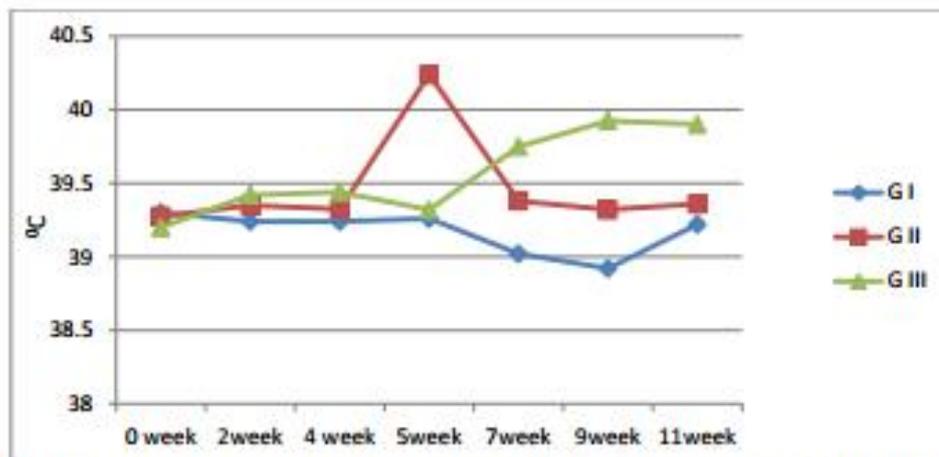


Figure (1) Average Temperature in Vaccinated and Control Lambs Groups

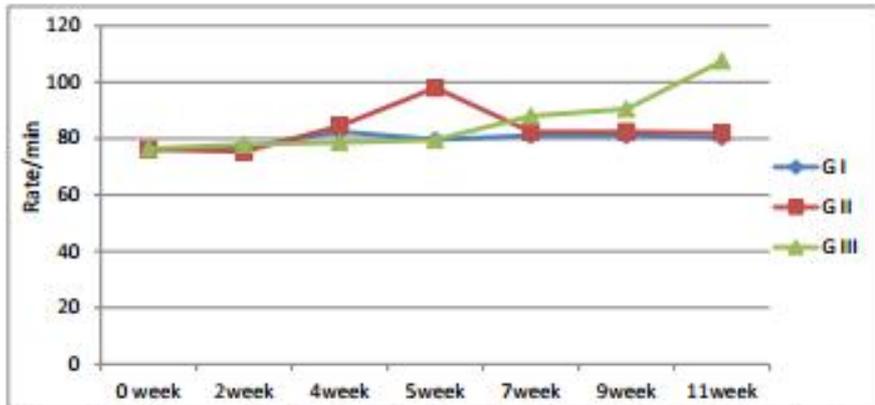


Figure (2) Average Pulse Rate in Vaccinated and Control Lambs Groups

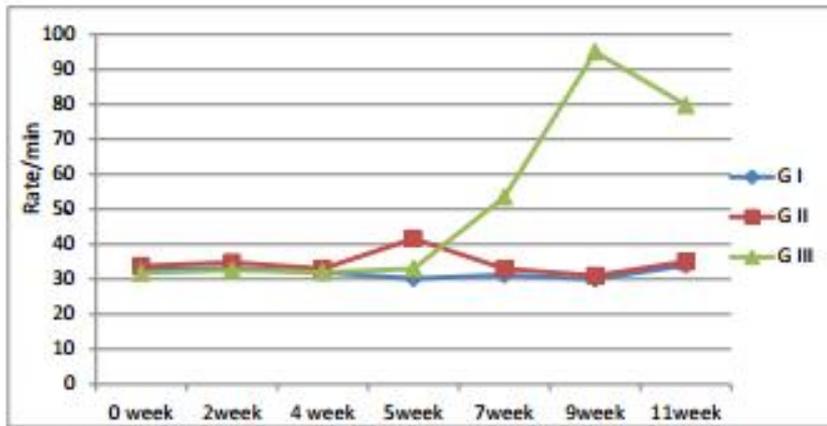


Figure (3) Average Respiration in Vaccinated and Control Lambs Groups

The Lambs in group III none vaccinated after challenged they revealed moderate elevation in average in temperature, pulse rate and respiration rate as showing in Table (1).

Table (1) Clinical Response (TPR) to Challenge in all Lambs Groups

Mean \pm SD			Groups
Respiration	Pulse	Temperature.	
31.2 \pm 5.215	80.5 \pm 5.727	39.02 \pm 0.497	GI
38.6 \pm 3.975	94 \pm 0.316	40.2 \pm 0.316	GII
54.6 \pm 5.702	112 \pm 3.162	40.62 \pm 0.130	GIII

The site of injection showed redness and induration and clinical signs persisted for 5 – 7 days. On the 5th day post challenged the lamb number one died while the other lambs showed inflammation and abscess formation at the site of injection.

Humoral Immune response

All lambs were negative on indirect ELISA and no antibody titer was recorded pre vaccination. The level of anti PLD IgG antibodies of each animal were determined and presented in Figures (4 and 5) as the absorbance values at 450 nm obtained using indirect ELISA. Two weeks after the first dose of vaccination the antibody titer developed in all vaccinated lambs (GII) with mean optical density (OD) values (0.426 \pm 0.26) while the control groups (GI and GIII) OD values were (0.096 \pm 0.013) and (0.093 \pm 0.01). The maximum titer was reached at the 8th week post vaccination (1.984 \pm 0.776) then the antibody titers begun to decrease to reach to (0.95 \pm 0.446) as in Figure (4)

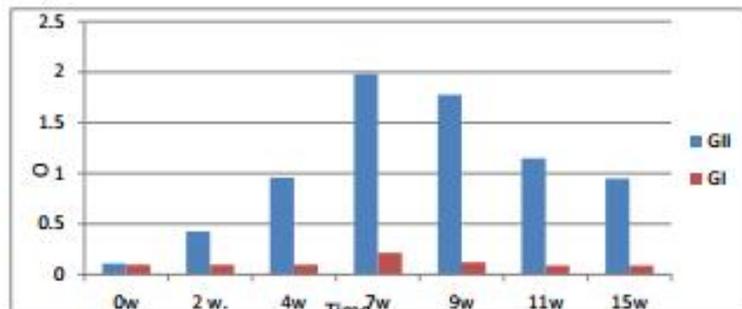


Figure (4). Mean of OD Represented IgG in Vaccinated Lambs Group (GII) and Control Group (GI)

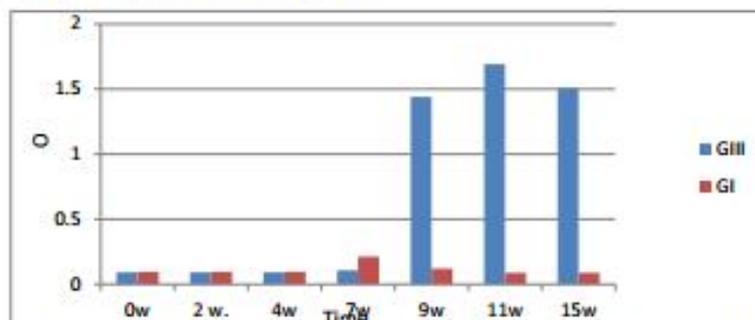


Figure (5) Mean of OD Represented IgG in (GIII) and Control Group (GI)

All lambs in GII showed no signs of infection during the experimental period. The lambs in GIII appeared rising antibodies titer two weeks post challenged and reached the maximum level within 5 weeks post challenged were the mean optical density was 1.691 ± 0.89 and then declined to 1.508 ± 0.09 after 3 weeks as in Figure (5).

Delayed Type Hypersensitivity- Skin test

All vaccinated lambs had positive skin reaction after intradermal injection of antigen which manifested by induration and thickening of the skin. Two weeks after the second dose of the case-bac vaccine, skin reactivity was determined in two groups. While negative control group GI did not show any skin reaction as in Figure (6) and Table (2).

Table (2) DTH-Skin Test Reaction in Vaccinated Lambs Groups

Time/hours				Skin thickness (mm)	Soluble Ag 0.5 mg/ml
72	48	24	0		
1.07-1.80	1.12-1.82	1.15-2.04	0.97-1.62	Range	Control negative group (GI)
1.518 ± 0.327	1.586 ± 0.317	1.62 ± 0.345	1.344 ± 0.369	Mean ± SD	
2.04-2.95	2.25-3.47	2.15-3.39	1.12-1.69	Range	
2.47 ± 0.420	2.87 ± 0.467	2.81 ± 0.537	1.49 ± 0.223	Mean ± SD	Vaccinated group (GII)
2.14-2.74	2.94-3.35	2.45-2.99	1.22-1.45	Range	
2.55 ± 0.278	3.07 ± 0.194	2.73 ± 0.673	1.36 ± 0.083	Mean ± SD	

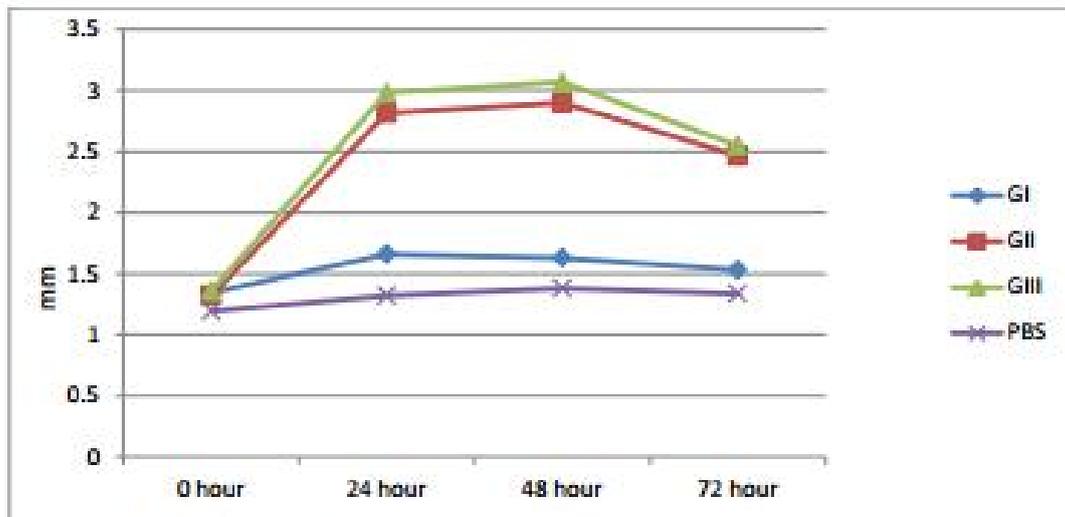


Figure (6) DTH-Skin Test Reaction in all Lambs Groups

DISCUSSION

The data of CLA incidence in sheep flocks in Iraq have not been recorded yet. Caseous lymphadenitis in sheep in Iraq is a disease which was not studied until now; and if it was done, with very limited and restricted works regarding isolation of *C. pseudotuberculosis* as an isolate, or in a group of isolates that were surveyed with other pathogens.

This study is the first one conducted using the commercial vaccine Case-Bac for vaccination of sheep in Iraq. Antibody levels due to CLA vaccine were detected using ELISA test as in many studies of serological tests (10; 11; and 12). Vaccination with Case-Bac vaccine resulted in a complete protection against challenge and these results coincide with full protection achieved by (8) and with (13). The study showed that there was fluctuation in individual animal responses to vaccination in which antibody titer differed from one lamb to another and the OD ranged from 0.409 to 2.409 on the 8th week post vaccination. This fluctuation may be due to the individual differences of immune status or may be due to persistent of other infection.

Vaccinated group (G II) showed increased in antibodies level (OD absorbance) after 3 weeks of the first dose of vaccine and reached the maximum level at the seventh weeks post vaccination. Similar finding has been reported by (13) while antibodies titer decreased after 3 weeks of challenge.

In unvaccinated control group (G III) antibodies increased 2 weeks after challenge and declined after 3 weeks as this fact agreed also with (13). Vaccination subcutaneously has been shown to induced humoral and cell mediated responses (14). Antibody titers of vaccinated sheep were measured by ELISA to evaluate the humoral response which induced by vaccine and play a role in protection against CLA (15 and 16). *C. pseudotuberculosis* well known as a facultative intracellular microorganism, so cell mediated immune response play a major role in protection and providing adequate protection against CLA (8).

In conclusion, this vaccine appeared to offer an excellent protection against the development of CLA in sheep and the vaccine could play an important role in the control of the disease in infected sheep flocks.

مرض التهاب الغدد التجمبي في الاغنام العراقية وتقييم التحصين باللقاح التجاري

طه ياسين غني و فيصل غازي حباشة
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الخلاصة

تضمنت الدراسة الحقلية 15 حملا. قسمت الحملان الى 3 مجاميع متساوية تحتوي كل مجموعة على خمسة حملان. لقت حملان المجموعة الثانية باللقاح التجاري Case-Bac واعطيت جرعتين بينهما فترة اسبوعين بينما بقيت حملان المجموعة الاولى كمجموعة سيطره سالبة. تم تعريض حملان المجموعتين الثانية والثالثة الى جرعة تحدي تحت الجلد 9×10^8 CFU تحتوي عترة ضارية من جرثومة وتدييات التدرن الكاذب. وتم قياس مستوى المناعه الخلطية في جميع المجاميع اسبوعيا، وكذلك تم قياس مستوى المناعه الخلويه باستخدام اختبار الجلد كما تم قياس درجة الحرارة ومعدل النبض ومعدل التنفس اسبوعيا. وكانت نسبة الحمايه من الاصابة التجريبية بجرعة التحدي في المجموعة الملقحة 100% بينما المجموعة الثالثة سجلت موت احد الحملان في اليوم الخامس من الحقن مع اصابة بقية الحملان بخراجات في منطقة الحقن.

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