Sudan J. Vet. Res. (2016), 31: 33-38. *sudanjvr.net*
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Protection Induced in Goats by *Corynebacterium pseudotuberculosis* Cell Wall Lysate and Toxoid Preparations

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ملخص البحث

جهز لقاحان من عترة محلية مختارة (CP41N) لوتدية السل الكاذب (*Corynebacterium pseudotuberculosis*) التى تسبب التهاب العقد الليمفاوية الجبنى (caseous lymphadenitis) فى الأغنام (الضأن) والماعز. يتكون اللقاح الأول من حُلالة جدار الخلية بجرعة 8مجم بروتين/ واحد مل حُلالة والثانى من حُلالة جدار الخلية زائداً الذوفان بجرعة 8 مجم بروتين/ واحد مل ذوفان (7 مجم بروتين/0.5مل). حقن كل لقاح تحت الجلد في مجموعة من الماعز (خمس حيوانات) بجرعة شهرية لمدة ثلاثة أشهر. أما المجموعة الثالثة (خمس حيوانات أيضا) فقد تركت بدون لقاح كمجموعة ضبط و مقارنة. حقنت جميع الحيوانات بعد ثلاثة أسابيع بعد الجرعة الثالثة للقاحين بجرعة إسقامية (1.2×10^5 وحدة تكوين مستعمرة بكتيرية / مل) عن طريق الوريد الودجي (واحد مل). ذبحت كل الحيوانات بعد شهر من الجرعة الإسقامية لفحصها بدقة بحثاً عن آفات المرض. وجد أن هنالك فرقاً جوهرياً ($P<0.01$) في خفض عدد آفات المرض في كل من حيوانات مجموعتي اللقاحين عن مجموعة الضبط و المقارنة. كما وجد أيضاً فرقاً جوهرياً ($P<0.05$) في خفض عدد آفات مجموعة اللقاح الثانى عن آفات المرض فى مجموعة اللقاح الأول. كانت نسبة الحماية لحيوانات مجموعة اللقاح الثانى و حيوانات اللقاح الأول مقارنة بحيوانات مجموعة الضبط و المقارنة في الأعضاء الداخلية [الرئة، الكبد، الطحال، الكلية، الثرب (غشاء الأمعاء الشحمي) و العقد الليمفاوية المنصفية و المساريقية] هى 65.2% و47.8 % على الترتيب. تعتبر هذه النتائج واعدة ومشجعة لتجريب اللقاح الثانى (الحُلالة زائداً الذوفان) لحماية حيوانات الحقل من المرض.

Summary

Two types of caseous lymphadenitis vaccines were prepared from a *Corynebacterium pseudotuberculosis* local strain CP41N that causes caseous lymphadenitis in sheep and goats. The first vaccine was a cell wall lysate (CW) which contained 8 mg protein/1ml and the second was a cell wall lysate of 8mg protein/1ml plus toxoid of 7mg protein/0.5ml. Three doses of each vaccine were injected s/c at one month interval in two groups of goats (five animals each). The third group was used as a non-vaccinated control. Three weeks after the last vaccination, all animals were challenged i/v with $1.2X10^5$ CFU/ml of *Corynebacterium pseudotuberculosis* (CP 41N). One month after the challenge, all animals were slaughtered and autopsied for caseous lymphadenitis lesions. Significant reduction ($P<0.01$) had occurred in number of lesions in animals vaccinated with first and the second vaccines compared with the controls. On the other hand, there was a significant reduction ($P<0.05$) in the number of lesions in animals vaccinated with the second vaccine compared with animals vaccinated with the first one. The protective efficacy conferred on internal organs (lungs, livers, spleens, kidneys, pericardia, omenta, mediastinal and mesenteric lymph nodes) from lesions, amounted to 65.2% and 57% for the second and the first vaccines, respectively. The results obtained were promising and encouraging to test CW lysate + toxoid vaccine for disease prevention in the fields.

Introduction

Corynebacterium pseudotuberculosis causes caseous lymphadenitis (CLA) in sheep and goats. The disease is characterized by abcessation of peripheral lymph nodes, in severe cases the visceral lymph nodes and internal organs may be involved (Maddy, 1953; Doty *et al*, 1964; Kimberling, 1988). The disease is distributed worldwide. In the Sudan, it was first diagnosed in Sinnar (Carr, 1914) and investigated in Omderman slaughterhouse and South Darfur (Amin, 1971; El gaddal, 1997; Musa, 1998). Once the disease is endemic, it is almost impossible to eradicate; antibiotic therapy is generally not effective because antimicrobials are unable to penetrate the encapsulated lesions. Identification of infected animals is difficult as internal abscesses escape detection (Brown and Olander, 1987). Vaccination seems to be the most suitable means for controlling the disease. Many trials had been made to produce vaccines against the disease in sheep and goats (Brogden *et al*, 1984; Lea Master *et al*, 1987; Brogden *et al*, 1990; Eggleton *et al*, 1991).

This study aimed at investigating the protection produced against caseous lymphadenitis in goats by CW lysate and CW lysate + toxoid vaccines.

Materials and Methods

Experimental animals

Fifteen Nubian goats of four to six-month-old, 9-16kg in bodyweight and were sero negative for *C. pseudotuberculosis* by the bacterial agglutination test, were bought from the local market. These animals were divided into three groups of five each. They were kept in pens, fed and watered *ad libitum* and observed for appearance of any clinical signs for five weeks before vaccination.

Bacteria and vaccines preparation

A local *C. pseudotuberculosis* strain CP41 N which has high cellular growth and good exotoxin production potentialities was chosen from other strains isolated from sheep and goats in South Darfur. This strain was used for preparation of toxoid and cell wall lysate as described by (Abdel Wahab, 2000).

Composition of the vaccines

One ml of cell wall lysate of *C. pseudotuberculosis*, that contained 8mg protein was mixed with saponin to give a concentration of 1mg saponin/ml vaccine. This constituted a cell wall vaccine no.1. Vaccine no. 2 was composed of the latter plus half ml toxoid containing 7 mg protein. Saponin was mixed with the two components to give a concentration of 1 mg/ml.

Vaccination programme

The vaccines were inoculated subcutaneously on the right side of each neck after shearing and disinfection of the injection site by alcohol. One ml of vaccine no.1 was injected into each goat of the first group whereas one and-half ml of vaccine no.2 was injected into each goat of the second group. Five goats were left as unvaccinated control. The second and the third doses of the vaccines were similarly injected at one-month interval each.

Challenge

Three weeks after the third dose of vaccination, a challenge suspension of 1.2 $X10^5$ CFU/ml of *C. pseudotuberculosis* was prepared and administered intravenously (1 ml) via the jugular vein, to each of the 15 experimental goats using sterile needles.

Post mortem examination

All animals were slaughtered four weeks after challenge. Parotid, mandibular, cervical, prescapular, prefemoral and superficial inguinal lymph nodes, the lungs, liver, spleen, kidney, heart, other abdominal viscera and internal iliac, tracheobronchial, and mesenteric lymph nodes were examined visually and by palpation. The lungs and the heart were removed from each carcass and placed in labelled plastic bags. Lymph nodes were incised at 1-cm apart. Detection of a lesion of CLA greater than 1mm was regarded as

positive. Specimens from the lesions were cultured onto Blood Agar.

Protective efficacy was calculated (Orenstein *et al,* 1985) as follows:

$$\text{Vaccine efficacy} = \frac{\text{attack rate of controls} - \text{attack rate of vaccinates}}{\text{Attack rate controls}} \times 100$$

Statistical analysis:

Comparisons between the experimental animals were carried out statistically, using Student-*t test*. The levels of significance applied to data were $P<0.05$ and $P<0.01$.

Results

Post mortem examination

Four animals died following the intravenous challenge, two from the control group on days 11 and 20 and two from the first group, on days 13 and 17. At slaughter, three of the five animals in the second group were found free from CLA abscesses in all organs. The other goats showed variable numbers of lesions (Table 1). Abscesses were seen in the lungs of control animals, in animals of the first group, and in two animals of the second group. In the liver, abscesses were observed in control animals, in four of the first group, and in two of the second group.

In the present study, both CW lysate and CW lysate + toxoid vaccines produced significant protection against CLA lesions ($P<0.01$) when compared with controls (Table 2). The protective efficacy increased significantly ($P<0.01$) when toxoid was added to the cell wall preparations compared to that produced by cell wall lysate vaccine alone (Table 2). There were no deaths due to acute infection in animals of the second group, whereas two animals of both the first group and the controls died after challenge. The deaths among the second group, the first and the controls were zero, 40% and 40%, respectively (Table 1). Only two animals of the second group developed CLA abscesses (40%), whereas in the first group and the controls all the animals were affected (100%). There was no complete protection of animals in the first group and the controls; all the animals had pyogenic lesions in variable numbers after i/v challenge with *C. pseudotuberculosis* CP41N. The rate of lesions of CLA in lungs, livers, spleens, kidneys, pericardia, omenta, and mediastinal and mesenteric lymph nodes in the first, second and control groups were 30%, 20% and 57.5%, respectively. The protective efficacy was 65.2% and 47.8% for CW lysate + toxoid and CW lysate vaccines, respectively.

Discussion

The results of this study show that CW lysate +toxoid produce better protection when compared with the CW lysate alone. They support the concept that cell-associated antigens and toxins play an important role in immunisation against *C. pseudotuberculosis* infection. The findings of this study also support the concept that pulmonary lesions are caused by haematogenous spread of *C. pseudotuberculosis*. In other studies, intradermal or intravenous inoculation resulted in mediastinal and pulmonary abscesses in sheep and goats (Brogden *et al*, 1984).

Table 1: Experimental challenge in vaccinated and control goats.

Parameter	**Non vaccinated animals (control group)**	**Animals vaccinated with cell wall lysate (first group)**	**Animals vaccinated with cell wall lysate+ toxoid (second group)**
	No/Total (%)	**No/Total (%)**	**No/Total (%)**
Deaths*	2/5 (40)	2/5 (40)	0/5 (0)
Animals with abscesses	5/5 (100)	5/5 (100)	2/5 (40)
Organs with abscesses:			
Lung	5/5	5/5	2/5
Liver	5/5	4/5	2/5
Spleen	4/5	1/5	1/5
Kidney	1/5	0/5	0/5
Pericardium	1/5	0/5	1/5
Omentum	2/5	0/5	1/5
Mediastinal L.N	3/5	2/5	1/5
Mesenteric L.N	2/5	0/5	0/5
Total**	23/40 (57.5%)	12/40 (30%)	8/40 (20%)
Protection %		**47.8%**	**65.2%**

* Goats which died also had abscesses.
** Total number of organs (lungs, livers, spleens, kidneys, pericardia, omenta mediastinal and mesenteric lymph nodes) for all five goats in the group.

Table 2: Comparisons of abscesses mean numbers in different organs in vaccinated and control goats after challenge (Student-*t test*).

A. Control group versus first and second groups:

Location	**Control group**	**First group**	**Second group**
Lung	45. 6	$9.6 \pm 5.7^{**}$	$2.2 \pm 5.2^{**}$
Liver	8.0	$4.2 \pm 1.0^{**}$	$0.8 \pm 1.11^{**}$
Spleen	2.0	$1.0 \pm 0.47^{N.S}$	$0.2 \pm 0.47^{**}$
Mediastinal L. N.	1.0	$0.4 \pm 0.22^{*}$	$0.2 \pm 0.2^{**}$
Kidney	0.4	$0.0 \pm 0.17^{*}$	$0.0 \pm 0.17^{*}$
Pericardium	1.2	$0.0 \pm 0.14^{**}$	$0.4 \pm 0.22^{**}$
Omentum	2.4	0.0±0.28**	0.6±0.33**
Mesenteric L.N.	0.4	0.0±0.17*	0.0±0.17**
Overall means of abscesses	**60.8**	**15.0±8.1****	**4.4±5.1****

* = Significant at 0.05; ** = Significant at 0.01; $^{N.S}$ = Non- significant

B. First group versus second group (caseous lymphadenitis involvements)

Location	**First group**	**Second group**
Lung	9.6	$2.2 \pm 2.6^{*}$
Liver	4.2	$0.8 \pm 0.7^{**}$
Spleen	1.0	$0.2 \pm 0.20^{**}$
Mediastinal L. N.	1.0	$0.4 \pm 0.22^{**}$
Kidney	0.0	$0.0 \pm 0.0^{N.S}$
Pericardium	0.0	$0.4 \pm 0.17^{*}$
Omentum	0.0	$0.6 \pm 0.17^{**}$
Mesenteric L.N.	0.0	$0.0 \pm 0.0^{N.S}$
Overall means of abscesses	**15.0**	**$4.4 \pm 5.1^{*}$**

* = Significant at 0.05; ** = Significant at 0.01; $^{N.S}$ = Non- significant

In the present work, no lesions were found in superficial lymph nodes and all lesions were in the lungs, livers, spleens, kidneys, pericardia, omenta, and mediastinal and mesenteric lymph nodes. In addition, acute toxication has led to the death of two animals in each of the first group and the controls. The widespread lesions and severity of infection might have been due to the intravenous inoculation of high challenge dose. Brogden *et al* (1984; 1990) used 3.1X 10^4/ml and 3.5X 10^4/ml CFU as intravenous doses, respectively, which appeared to be the threshold dose. Moreover, the weight of the experimental goats in this study ranged from 9-16 kg compared to about 25 kg lambs used by others elsewhere. On the other hand, in this study the 12 hr broth culture used was not washed or dispersed and the surface lipid content of the challenge strain was not determined. It was stated that agglutination of bacterial cells due to presence of surface lipid might had facilitated the concentration of exotoxin *in vivo* (Burrell, 1978). More virulent strains possessed more extractable lipids than attenuated strains (Jolly, 1966; Ellis *et al*, 1990). From this study, it could be assumed that the use of a large unwashed and non-dispersed cells challenge dose had exaggerated the burden imposed on the immune system which explain the widespread and the high number of internal lesions.

Lea Master *et al* (1987) have believed that vaccination doses do not affect the lesion size only, but affect the establishment of virulent organisms in the host body. Once the organism is established, lesions develop at the same rate in the vaccinated and non-vaccinated lambs and this was indicated by the similarity in lesion size between the groups. Our results show that in vaccinated animals, lesions are variable in size and are less in numbers compared to those in the control group. The results of this study agree with Burrell (1978) who has postulated that several factors including the initial number and rate of multiplication of the organisms inside the host influence the size of such lesions. According to the results of this study, it is recommended to vaccinate large groups of goats with WC + toxoid in order to validate its use.

Acknowledgements

The authors would like to thank Mr. S. A. Noga for technical assistance. The permission of the Director General of Animal Resources Research Corporation to publish this article is acknowledged.

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