Sudan J. Vet. Res. (2009), **24**: 11-15.
With 1 figure in the text. *www*. sudanjvr.net.

Production of Abscess (Morel's) Disease Vaccine by the IBT Bioreactor Technology

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ملخص البحث

أثبت إستنبات البكتريا العنقوديه نويع اللاهوائيه المسببه لمرض الدمامل في الضأن بتقنية المخمر بواسطة نظام الإستنبات المستمر فعالية وأعطى نمواً بكتيرياً جيداً ولقاحاً ذا كفاءه عالية. الوسط الامثل للنمو لهذه التقنية هي أس أيدروجيني 6.4 ، ودرجة حرارة 37 ° C مئويه ونسبة تخفيف الوسط الغذائي إلي 0.04%. نتج عن هذا الوسط حافظة بكتيرية متماسكة ومتطوره خصوصاً من الإستنبات المستمر للبكتيريا. لقد أستخدم في هذه الدراسة نوعان من اللقاح بنسب تركيز مختلفة للخلايا والذوفان ، لقاح مخلوط بنسبة 60% خلايا و40% ذوفان وآخر بنسبه 50% خلايا و50% ذوفان كما إستعمل أيضاً لقاح منتج من الحصاد الكلي الميت بواسطة الفورمالين . اللقاح المنتج من الحصاد الكلي أعطي 60% وقاية بينما أعطي نوعا لقاح الخلايا والذوفان وقاية بنسبة 85.8 و 78.6% على التوالي.

Summary

Cultivation of S*taphylococcus aureus* subsp. anaerobius (the causative agent of abscess disease of sheep), in the IBT bioreactor using continuous culture system gave good growth with high quality vaccine. The optimum growth conditions for continuous culture were pH 6.4, temperature 37^{0}C and a dilution rate 0.04%. The capsule was detected in both the static and the continuous cultures, but was more developed in continuous culture. The bioreactor culture was inactivated, filtered and used to prepare two types of vaccines with different concentration of cells and toxoid (60% cells + 40% toxoid and 50% cells + 50% toxoid), which gave 85.8% and 78.6% protection, respectively whereas, a formalinized whole culture vaccine gave 60% protection.

Introduction

Abscess (Morel's) disease (sheep abscess syndrome), is a specific lymphadenitis that affects young sheep in the Sudan and causes great economic losses. It was first reported in the Sudan by Hamad (1989). Trials of producing a vaccine for the the disease were initiated by Elsanousi and Abass in 1988 (personal communication) using a formularized killed bacterin of *S. aureus* subsp. anaerobius to protect young lambs. The vaccine reduced the prevalence rate of the disease by 65% and moderately decreased the abscess size in vaccinated lambs that acquired the infection. Rodwan (1996) tried a vaccine composed of formularized whole culture, toxoid and capsule antigens against abscess disease in sheep that resulted in 96% protection.

The technology of the bioreactor has successfully been used for production of bacterial vaccines, eg, clostridiosis (Böhnel, 1986; Röth, 1986; Babiker, 1991; Schaper, 1991), pasteurellosis (El Bashir, 1993), mycoplasmosis (Häusser, 1989), brucellosis (Sonnenberg, 1993) and some viral vaccines (Niβlein, 1993).

The aim of this study was to produce a vaccine for abscess disease in sheep by the bioreactor technology using the locally isolated strain of Rodwan (1996).

Materials and Methods

Seed strain:

A local *S. aureus* subsp. anaerobius strain KHR11 (Rodwan 1996) from sheep with abscess disease] was used for cultivation in IBT bioreactor; a system constructed to suit the conditions of the tropical countries.

Media used:

A modified Reinforced Clostridial Medium (RCM) derived from original RCM (Oxoid, CM 149) was used as a nutrients supply in twenty litres flask autoclaved in a nutriclave as separate ingredients and mixed in the flask. Brain Heart Infusion (BHI) (Oxoid, CM225), was used for seed preparation.

Bioreactor:

The IBT Gottingen bioreactor consists of the fermenter vessel that controls the pH, redox potential and temperature. The medium flask was connected with the nutriclave and used as a medium reservoir and the thermocirculator for regulating the temperature.

Cultivation of *Staphylococcus aureus subsp.* anaerobius in the Bioreactor:

Forty eight hours culture of *S.aureus* subsp. anaerobius strain KHR11 grown in 5% Co_2 at 37^0C was inoculated into the bioreactor vessel after sterility testing of the bioreactor. Series of experiments were carried out to determine the optimal growth conditions, i.e. pH, temperature and dilution rate that gave good bacterial growth of high viable bacterial count, well-developed capsule and toxin production. According to these experiments, a continuous bacterial growth was carried out under the required optimal conditions determined. The culture was inactivated by 3% formal-saline, incubated at 37^0C and then filtered.

Filtration of the formalinized culture:

The filtration system aimed to produce concentrated vaccine components out of the formalinized fermenter broth. This was done by two consecutive phases of filtration as follows:

The bacterial cells and their fragments were separated in phase one by Enka filter, the medium contained media fragments, toxoid and other metabolic products. Filtration was continued until the whole bacteria-free fluid was obtained. The retained bacterial cells in the filter and silicon tubes were flushed back into the original flask.

The filtrate obtained from phase one was filtered by recycling it inside the second filter (Fresenius, Model SPS 600). The lower molecular weight proteins and components, formalin and water were passed through the filter pores and discarded. The rest that remained in the flask was considered the toxoid.

Preparation of the Vaccines:

The concentrated washed cells and the toxoid were used to prepare the vaccines using 3% formal saline as a diluent. The vaccines used were 60% cells + 40% toxoid, 50% cells + 50% toxoid and formalinized killed whole culture that was taken before filtration.

Safety Test of the Vaccine:

Fifteen Hammary lambs, 1-2 year-old and, 19-20 kg. l.b.wt. were purchased from Abuzeid Animals Market in Omdurman, Khartoum State, and were divided into 3 groups of 5 lambs each. Each group was vaccinated with 2 ml (double dose) of each vaccine. Rectal body temperature and reaction at the inoculation site were recorded for all groups for 7 days following vaccination.

Vaccination of Sheep:

Twenty-eight Hammary lambs, 1-2-year-old, 19-20 kg.b.wt, were purchased from Abuzeid Animals Market in Omdurman, Khartoum State. They were examined thoroughly for external abscess and divided into 4 groups of 7 lambs each. Group 1 was vaccinated with 60% cells + 40% toxoid, group 2 recieved 50% cells + 50% toxoid, group 3 with formalinized killed whole culture vaccine, while group 4 was kept as non-vaccinated control. Each sheep was inoculated subcutaneously with 1ml of each vaccine at the right side of the neck region. The sheep were kept under observation for a period of 30 days before being challenge.

Potency Test:

The sheep were challenged 30 days post-vaccination. The challenge dose was 750,000 bacterial cells of *S. aureus* subsp. anaerobius strain KHR 11 injected subcutaneously at the left side of the neck region. The rectal body temperature and the reaction at the site of inoculation were daily recorded; the reaction was measured in mm. The sheep were euthanized two weeks PI.

Results

Optimum physical parameters for bacterial growth:

Capsule and toxin production in the bioreactor:

The best viable count ($1x10^{10}$cfu), best total count (2.559 absorbance at 550 nm), the best redox potential (-235.4/mV) and the well-developed capsules were obtained at pH 6.4, temperature 37^0C and 0.04% dilution rate.

Filtration:

After filtration highly purified and concentrated toxoid (detected by protein determination) and concentrated cells were obtained. The culture suspension was concentrated ten times. The capsule also was detected after filtration.

Safety and Potency tests:

All vaccines were well tolerated by the vaccination study and no systemic or local reactions at the inoculation site and rectal body temperature was normal in all vaccinated sheep.

The potency test following slaughter of vaccinated animals have no reaction, while others showed reaction and with time it became small or completely disappeared. In non-vaccinated control animals, most animals developed large abscesses. In general the inflammatory reactions in the vaccinated sheep occurred later in comparison to the non-vaccinated control sheep. The protection percentage produced by vaccination was calculated as follows:

$$\% \text{ sheep protected} = \frac{\text{Number of protected animals}}{\text{Number of animals vaccinated}} X100$$

Figure 1 shows the protection induced by each vaccine type, Type one (60% cells + 40% toxoid) was the best and gave 85.8% protection, followed by type two (50% cells +50% toxoid) which gave 78.6% protection, while type three (whole culture) gave (60%). protection.

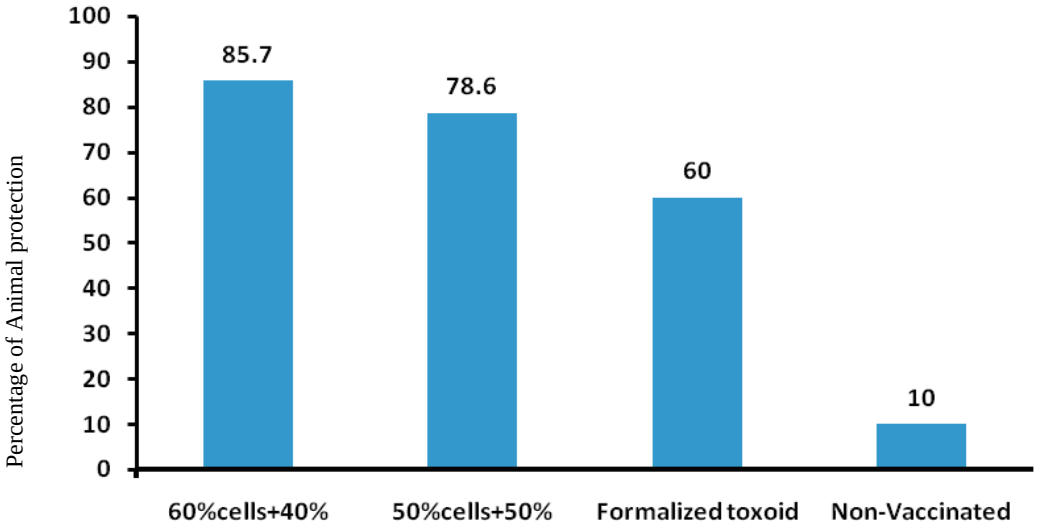

Fig. 1: Percentage of animals protected by the 3 different vaccine Types used.

Discussion

The cultivation of *S. aureus* subsp. anaerobius in the IBT bioreactor confirmed the superiority of the continuous culture over the static culture. The capsules in the continuous culture were better developed and thicker than those in the static culture. The filtration of the formalinized culture broth was done to reduce the amount of formalin, water and the low molecular weight proteins, which might induce undesirable immune responses or allergic reactions. It also facilitated the separation of toxoid from cells and concentration of both components to the desired volume.

The optimum pH, temperature and dilution rate for the growth of *S. aureus* subsp. anaeroius in IBT bioreactor were 6.4, 37^{o}C and 0.04%/h^{-1}, respectively.

Since there is no available literature concerning the continuous culture of *S. aureus* subsp. anaerobius in the bioreactor, this study represents the first trial to cultivate this organism in the bioreactor.

The use of cells and toxoid vaccines is based on the theory that combination of cells and toxoid will generate immunity in vaccinated sheep to both components, provides an additional effect for the prevention *S. aureus* subsp. anaerobius infection. Although high inoculum was used for challenge (750,000) compared with the minimum abscess-causing dose which is 1200 bacterial cells (Rodwan, 1996), a good protection was obtained by the three vaccines used.

The vaccine comprising 60% cells + 40% toxoid, gave higher percentage rate (85.8%) and no abscess was detected at the inoculation site; skin reaction was observed with no involvement of the upper lymph nodes. These results are similar to those obtained by Rodwan (1996) who used a combination of a formalinized whole culture, toxoid and capsule as a vaccine for protection of sheep against abscess disease. Watson (1988) found that killed *S. aureus* cells with toxoided staphylococcal β-haemolysin and dextran sulphate as adjuvant, induced immunity to ewes after an intramammary challenge. Moreover, Watson (1992) used

killed cell-toxoid-adjuvant *S. aureus* vaccine for dairy heifers, the organism was grown under conditions that induced the expression of pseudocapsule. After challenge by an intramammary infusion, the vaccinated heifers were more resistant to clinical mastitis than the controls.

The vaccine comprising 50% cells + 50% toxoid also gave a good level of protection (78.6%), however, there was an abscess at the inoculation site in one sheep, skin reaction in several ones and involvement of the right prescapular lymph node in one sheep.

The formalinized whole culture vaccine that gave 60% protection was similar to that reported by ElSanousi and Abass (personal communication).

Acknowledgements

The assistance of Dr. Frank Gessler is greatly appreciated. Thanks are also extended to the Director of the Central Veterinary Research Laboratories Centre (CVRLC) and the Director General of Animal Resources Research Corporation (ARRC) for permission to publish this article.

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