SOME FACTORS AFFECTING THE PRODUCTION AND BIOLOGICAL ACTIVITY OF CLOSTRIDIUM SEPTICUM ALPHA TOXIN USED IN VACCINE PREPARATION

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ABSTRACT

This study was done to obtain high yield of Clostridium septicum alpha toxin through using different media, different pH level and activation of protoxin to active toxin by proteolytic enzyme were applied. Toxin yield was markedly improved by cultivation of C. septicum in production media containing meat particles, pH (7.5) and activation the protoxin. The prepared vaccines from these conditions gave higher antibody titer than ordinary one in vaccinated sheep.

KEY WORDS: Clostridium Septicum, Sheep, Toxin, Vaccine.

1. INTRODUCTION

Clostridium septicum (C. septicum) is a Gram positive, motile, spore forming anaerobe; that has been implicated as a cause of malignant edema in animals [5, 31]. The organism produces several extracellular factors that include deoxyribonuclease, hyaluronidase, neuraminidase and alpha toxin [25]. Alpha toxin is the major virulent factor with hemolytic, lethal and necrotizing activities [3, 12]. It is secreted as water soluble protoxin that is activated by proteolytic cleavage of an amino terminal propeptide by such cell surface proteases as furin or furin-like proteases [3, 12, 30]. C. septicum gangrene generally exhibit a poor prognosis due to the fulminant nature of the disease and the fact that early diagnosis is difficult without early treatment and the mortality rate is near 100% [18]. The severity and acuteness of clostridial toxaemia make the treatment of affected animal very difficult. Thus controlling the disease by active immunization is of considerable importance.

Active immunization depends on different factors mainly the antigenic characters of microbes and their toxins [4, 24]. The amount of total lethal toxin produced by C. septicum depends on the strain [7, 28], the medium [29] and the cultural conditions [8]. The adjustment of pH is important for producing maximum yield of toxin [20, 21]. In this respect [14, 16] found that increase in toxin production is by increasing glucose concentration in the media and manually adjusting the pH.

The efficient clostridial vaccines depend mainly on the amount and quality of the specific antigens [4, 24]. Since the preparation of good toxoid is dependent in part on the production of high titers of toxins grown under laboratory conditions, this study was under taken with the purpose of examining in some detail the requisites for growth and production of C. septicum toxins and preparation of
vaccines (polyvalent and bivalent) under these conditions and evaluate these vaccines in sheep.

2. MATERIAL AND METHODS

2.1. Determination of the optimum conditions required for maximum growth and toxin production for *C. septicum* alpha toxin:

2.1.1. Culturing on different media as:
- a. Peptone media.
- b. Peptone media + meat particles.
- c. Thioglycolate media.
- d. Tryptone soya broth.
- e. Brain heart infusion broth.

2.1.2. Growth with different pH values:
 Cultures of *C. septicum* were adjusted at pH 6.5, 7, 7.5 and 8.

2.1.3. Activation of protoxin:
 The activation is done by addition of 1 gm of trypsin (1/250) to 1000 ml of culture of *C. septicum* for 1 hour according to Hang Ombe et al. [12].

2.2. Determination of toxicity and haemolytic activity:
 Determination of Minimum Lethal Dose (MLD) of toxin of prepared culture was estimated according to British Pharmacopoeia [6]. The highest dilution of sample causing death of mice within 24 hours was designated as one lethal dose. Determination of hemolysin activity was applied according to Moussa [19].

2.3. Vaccine preparation:
 Four batches of vaccine were prepared:

1. **Polyvalent vaccine:** the currently prepared polyvalent vaccine which consists of equal amount of different clostridial antigens (toxoid of *C. perfringens* types A, B and D, *C. septicum*, *C. novyi* and whole culture of *C. chauvoei* and tetanus toxoid was added as 25 Lf/dose). The vaccine was prepared according to Gadalla et al. [11] and tetanus toxoid was prepared according to Rijks [22].

2. **Polyvalent vaccine:** the same vaccine as vaccine number one but contained treated *C. septicum* toxoid (media, pH and activated toxoid).

3. **Bivalent vaccine:** blackleg and gas gangrene vaccine consists of (*C. chauvoei* and *C. septicum* toxoid) in a ratio of 4:1 respectively. The vaccine prepared according to Gadalla et al. [11].

4. **Bivalent vaccine:** the same vaccine of number three but using treated *C. septicum* toxoid.

All vaccines were inactivated by formalin at 0.5% and alum at 1% was used as adjuvant.

2.4. Test for purity and safety of the prepared vaccine:
 The prepared vaccines were subjected to sterility and safety tests before using them in immunization of sheep according to European Pharmacopoeia [10].

2.5. Vaccination of sheep:

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Type of vaccine</th>
<th>Dose (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Polyvalent clostridial vaccine</td>
<td>3</td>
</tr>
<tr>
<td>II</td>
<td>Polyvalent clostridial vaccine containing treated <em>C. septicum</em> toxoid</td>
<td>3</td>
</tr>
<tr>
<td>III</td>
<td>Bivalent blackleg and gas gangrene vaccine</td>
<td>3</td>
</tr>
<tr>
<td>IV</td>
<td>Bivalent blackleg and treated <em>C. septicum</em> toxoid vaccine</td>
<td>3</td>
</tr>
</tbody>
</table>

(9-12 months old) were vaccinated subcutaneously; each animal received two doses 4 weeks apart as shown in the following table (1)

Table 1 Groups of sheep vaccinated with different prepared clostridial vaccines

Blood samples were collected from all groups before vaccination and 2 weeks after the second dose. Sera of each animal were tested for determination of the
antibody titer by using the serum neutralization test in white Swiss mice for alpha antitoxin of *C. septicum* [9].

3. RESULTS AND DISCUSSION

Apparently the most urgent problem in active immunization against most important clostridial diseases by the administration of toxoids, is the discovery of an improved method for the preparation of much more potent toxin [15]. Production of high yield of bacterial toxin is very important in the preparation of effective toxoid for purpose of immunization. In this study we try to cultivate *C. septicum* on different media at different pH levels and used proteolytic enzyme for activation of protoxin to obtain maximum toxin production.

Regarding to the influence of different media on production of *C. septicum* alpha toxin as show in table (2), it revealed that peptone media containing meat particles supported more production of alpha toxin (MLD and haemolysis) of *C. septicum* than other media. Production media represented a complex medium providing good conditions for growth and toxin production as stated by Jayko and Lichstein [17]. Alpha toxin is governed by the presence of high molecular weight peptide of peptone content in media for the production of this toxin [25]. Meat particles confirmed good growth reliably adequate production of alpha toxin.

**Table 2 Influence of different media on production of *C. septicum* alpha toxin**

<table>
<thead>
<tr>
<th>Media (pH 7.5)</th>
<th>MLD/ml of <em>C. septicum</em> alpha toxin</th>
<th>Hemolysin activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone media</td>
<td>60</td>
<td>1/16</td>
</tr>
<tr>
<td>Peptone media with meat particles</td>
<td>80</td>
<td>1/32</td>
</tr>
<tr>
<td>Thioglycolate media</td>
<td>50</td>
<td>1/8</td>
</tr>
<tr>
<td>Tryptone soya broth</td>
<td>40</td>
<td>1/4</td>
</tr>
<tr>
<td>Brain heart infusion broth</td>
<td>50</td>
<td>1/8</td>
</tr>
</tbody>
</table>

Respecting to the effect of optimum pH value of growth media Table (3) the result showed that the pH (7.5) was the optimum for maximum synthesis of alpha toxin (MLD and hemolysin) of *C. septicum*. This result suggested that the release of toxin from cells may be pH dependant. These results were parallel to those obtained by [1, 9, 11, 16, 26]. On the other hand, Hang Ombe et al. [13] stated that pH may exert an important role in the activity of *C.septicum* alpha toxin and found that anaerobic condition required for optimal growth of *C.septicum* produced on acid pH which in turn may enhance the activity of alpha toxin and low pH in an anaerobically environment might favor the degree of alpha toxin lethality.

**Table 3 Results of Studying the Optimum pH value of peptone media with meat particles for maximum alpha toxin production of *C. septicum***

<table>
<thead>
<tr>
<th>pH</th>
<th>MLD/ml of <em>C. septicum</em> alpha toxin</th>
<th>Hemolysin activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.5</td>
<td>50</td>
<td>1/8</td>
</tr>
<tr>
<td>7.0</td>
<td>70</td>
<td>1/16</td>
</tr>
<tr>
<td>7.5</td>
<td>80</td>
<td>1/32</td>
</tr>
<tr>
<td>8.0</td>
<td>50</td>
<td>1/8</td>
</tr>
</tbody>
</table>

From the results obtained in table (4) it was found that the active toxin gave higher level of MLD than protoxin. *C. septicum* produces and secretes its toxin in the logarithmic phase of growth, the toxin produced as protoxin, and its activation by the proteolytic enzyme gave more potent toxin [13]. An activation of protoxin to an active toxin leads to a pore of approximately 1.3-1.6 nm in the diameter of mammalian cells plasma membrane [2, 3]. In erythrocytes pore formation by alpha toxin induces selective membrane permeabilization to penetrate small ions, causing hemolysis [3, 23].

**Table 4 Comparison of active and none active protoxin of *C. septicum***

<table>
<thead>
<tr>
<th>Toxin</th>
<th>MLD/ml of <em>C. septicum</em> alpha toxin</th>
<th>Hemolysin activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protoxin</td>
<td>80</td>
<td>1/32</td>
</tr>
<tr>
<td>Active toxin</td>
<td>100</td>
<td>1/64</td>
</tr>
</tbody>
</table>

*C. Septicum cultivated in peptone media containing meat particles at pH 7.5*
The results in table (5) showed that immune response of sheep to treated *C. septicum* was higher than none treated toxoid in polyvalent and bivalent vaccines. The antibody titer of *C. septicum* in animals group vaccinated with bivalent vaccine was higher than those vaccinated with *C. septicum* in polyvalent vaccine. The variation in immune response between *C. septicum* in bivalent vaccine and in polyvalent vaccine may be attributed to what is called interference between antigens as claimed by Sterne et al. [27] who reported that immune response to a combined vaccine was lowered than to the components individually.

<table>
<thead>
<tr>
<th>Animals</th>
<th>Type of Vaccine</th>
<th>Pre-vaccination Titer</th>
<th>Post-vaccination Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>PVV</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Group II</td>
<td>PVVT</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Group III</td>
<td>BVV</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Group IV</td>
<td>BVVT</td>
<td>0</td>
<td>6</td>
</tr>
</tbody>
</table>

*Alpha anti-toxin titer of C. septicum 2 weeks post 2nd vaccination. PVV: Polyvalent vaccine. PVVT: Polyvalent vaccine containing treated C. septicum toxoid. BVV: Bivalent vaccine. BVVT: Bivalent vaccine containing treated C. septicum toxoid.*

From these results it could be concluded that using production media containing meat particles at pH 7.5 and activation of protoxin was essential for maximum yield of *C. septicum* alpha toxin for preparation of potent vaccine.

4. REFERENCES


Factors affecting C. septicum alpha toxin vaccine


بعض العوامل المؤثرة على إنتاج والنشاط الحيوي لسم الألفا الخاص بالكلوستريديم سبتكم المستخدم في تحضير اللقاح

د/ مجدى مصطفى السحمي
معهد بحوث الأمصال واللقاحات البيطرية

الملخص العربي

تم دراسة الظروف المثلى لزراعة عطرة الكلوستريديم سبتكم في أوساط غذائية مختلفة ودرجات الأس الهيدروجيني المختلفة وتنشيط السم من سم حام إلى سم نشط، وقد وجد أن الوسط الغذائي الذي يحتوي على حبيبات اللحم ودرجة الأس الهيدروجيني 7.5 والسم النشط أعطت أعلى معدل من السمية ويتقريب تلك المعدلات المثلى في تحضير اللقاح أعطت استجابة مناعية جيدة في مصل الأغنام المحصنة بها عن تلك المحصنة باللقاح المعتاد.

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