Short communication:
Serological Tests for Abscess Disease

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أجريت هذه الدراسة لتبنى إختباراً مصلياً لتشخيص مرض الدمامل بإستخدام مستضد الخلية الكاملة أو مستضد الذوفان و أمصال الحيوانات المصابة طبيعيا أو تجريبيا. الإختبارات المصلية التقليدية المستخدمة هي تراص الأنبوب و تراص البطاقة و الترسيب في هلام الأجار. أظهرت جميع هذه الإختبارات تراصاً أكيداً مع الأمصال الموجبة. إختير إختبار البطاقة إختباراً أمثل. وجدت 41 عينة موجبة من أصل 50 عينة مصل جمعت عشوائيا من حيوانات مصابة طبيعيا. كما أختبر أيضاً إختبار تراص الأنبوب لقياس درجة الإستجابة المناعية.

This study was conducted to adopt a serological test for sheep abscess disease diagnosis using whole cell or toxoid as an antigen, and serum samples from naturally or experimentally infected animals. The employed conventional serological tests included tube agglutination test (TAT), card agglutination test (CAT), and gel diffusion test (GDT). All tests showed agglutination with positive sera. A total of 50 sera randomly collected from naturally infected sheep was tested, 41 were found positive. TAT was chosen to monitor the immune response of the disease. CAT was chosen as test of choice.

Abscess disease is a disease of sheep and goats caused by *staphylococcus aureus* subsp *anaerobius*. It is characterized by the formation of suppurative lesion that is typically located in superficial lymph nodes (De La Fuente *et al,* 1985). The disease is endemic in nature with high morbidity and does not cause death (Bajmocy *et al,* 1984). It is seen in animals of good health which are being fattened (Aynaud, 1928). When an abscess becomes mature it ruptures and expels thin greenish pus and healing takes place after long time (Bajmocy *et al,* 984). Related work had already been done in the Sudan by some researchers (Bakhiet, 1996; Mohammed, 1996; Rodwan, 1996; Salih, 1997; Elhag, 2002).

The disease is diagnosed by only one test, plaque formation test, which is time-, money- and labour-consuming test. So other simple, cheap and easy to perform serological test(s) must be developed to diagnose abscess disease and to monitor the immune response.

Three local breed male sheep at age of six month and of 9-14 Kg bodyweight were purchased from the local market. They were free of abscess and other abnormalities. Sera were collected from apparently naturally-infected sheep showing superficial abscesses.

Staphlococcus aureus subsp *anaerobius* (ATCC35844) was cultured onto modified Brain Heart Infusion Agar (MBHIA) and incubated at 39 °C in a candle jar. The 24 hour growth was harvested with normal saline. The culture suspension was centrifuged at 3000 rpm for 15 minutes. The packed bacterial cells were washed two times in normal saline solution before they were re-suspended.

The afore-mentioned prepared whole cell antigen was centrifuged at 3000 rpm for 15 minutes. Each gram of cells was suspended in 20 ml of phenol saline; then 1ml of Rose Bengal solution was added to each 30 ml of this suspension and mixed with a magnetic stirrer for 2 hrs. The stained antigen was removed by centrifugation and 7 ml of normal saline was added to each gram of the packed stained cells.

A single colony of *S. aureus* subsp *anaerobius* (ATCC35844) was cultured into 5 ml modified Brain Heart Infusion Broth. The culture was incubated at 39Cº in a candle jar for 24 hrs in an inclined position, then the broth culture was centrifugated. The clear supernatant

was decanted into sterile bottles to which formalin was added and left for a month to make the toxoid.

A dose of 1.2x 10^4 colony forming unit (CFU) was inoculated subcutaneously in three sheep in day 0. Then after 28 days sera were collected and a second dose of 2.1x 10^5 CFU ml was inoculated. Then in day 42 sera were collected and a dose of 2.1x10^5 was inoculated. In day 49 sera were collected too.

Each serological test was performed employing ten randomly collected sera from naturally–infected sheep showing superficial abscesses. The test of choice was tried employing 5 sera collected randomly from the above–mentioned sera.

For tube agglutination test, double dilution of antisera in normal saline was made. Then 1 ml of the antigen was added to each tube. The mixture was incubated at 39 °C overnight, a negative control tube consisted of normal saline and the antigen was set. The tubes were examined visually for agglutination and clearance of supernatant.

Equal volumes of stained antigen and serum (10 µl) were placed on a card, mixed and spreaded over the tear-drop area; the test was read immediately.

In gel diffusion test (GDT), a pattern of wells was punch in the agarose gel using a gel cutter consisted of peripherally equidistantly punched six well surrounding a central well. A volume of 30 µl of each serum sample collected from sheep was placed in a peripheral well and the toxoid in the central well. Plates were incubated in a humidified chamber at room temperature for 24-48 hrs. The test was read against an illuminated chamber, clear precipitin lines were recorded as positive result.

The test showed a clear line with positive sera collected from sheep infected with abscess disease after 24 hrs.

Agglutination was clear and immediate in sera collected from abscess disease suspect sheep. Forty-one samples were positive, while nine samples were negative. Sera collected from sheep infected with abscess disease showed clear agglutination and clear supernatant after 24 hours.

Previous trials to develop simple and reliable test(s) failed because poor antigen was used. In this study good protective antigen was tried. Good antigen for the pathogen in question requires cultural conditions simulating those in the natural host.

Since *S. aureus* subsp *anaerobius* is host specific, special attention was paid to produce good antigen through the induction of virulence factors (Bakhiet, 1996). Determination of their optimum value of a particular environmental growth factor (temperature-pH-atmosphere-water activity or nutrients) is influenced by the other environmental growth factors which all should be simultaneously at their optimum values for the organism being tested. If all other factors are not simultaneously at their optimum values, the optimum value of that particular factor will be erroneous. Cultural conditions simulating those of the natural host stimulate production of protective antigens (Bakhiet, 1996).

In sheep the mean values of blood carbon dioxide and oxygen tensions are 5% and 4%, respectively (Saleh et al, 1990). These values are taken as the natural environmental carbon dioxide and oxygen tensions values for production of virulence factors. Carbon dioxide tension was increased by incubation under high carbon dioxide concentration and by incorporation of bicarbonate in the medium.

S. aureus capsule was promoted by incubation under an increased carbon dioxide tension (Fournier *et al,* 1984). Furthermore, *S. aureus* beta toxin production was stimulated by increasing carbon dioxide tension.

Immunization with bacterins prepared from *S. aureus* cell grown on conventional media does not confer protection (McDowell and Watson, 1974) whereas that with live *S. aureus* vaccine cells grown *in vivo* provides a considerable degree of protection (Watson, 1984).

S. aureus cells grown *in vivo* or in conditions which are supposed to simulate those *in vivo* express additional surface antigen when compared with cell grown in conventional media (Watson, 1992).

The micro capsule produced *in vivo* comprises hexose and uronic acid. Immunization with a bacterin prepared from cells grown in conditions which are supposed to simulate those in vivo does not offer protection unless dextran sulphate is added (Watson, 1992).

Dextran sulphate is a polymer of sulphate hexose; *S. aureus* dextran sulphate inoculum mounts a significantly greater anti- *S. aureus* IgG_2 response than does that of *S. aureus* alone (Kerlin and Watson, 1987). This suggests that sulphate hexose may act as a signal stimulus for IgG2 at the amino terminal domains of the B cells.

Currently, it may be recommended that CAT could be used as screening test and TAT could be used to monitor the immune response of vaccination since it can interpret the latter in form of titre. These tests are simple and economic and thus can replace plaque formation test which is time-, labour- and money-consuming.

Further studies are needed to develop non-conventional tests by employing values of some parameters (temperature and pH) and/or antigens used in these conventional tests.

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