

FAILURE OF AN *ACTINOMYCES PYOGENES* VACCINE TO PROTECT SHEEP AGAINST AN INTRAVENOUS CHALLENGE

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ABSTRACT

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The immunity conferred by an *A. pyogenes* bacterin-toxoid was evaluated in sheep, using an intravenous challenge system. Three sheep were vaccinated and 3 served as controls. The vaccinated sheep were not protected against pyogenic conditions. High antitoxin levels were induced by vaccination but could not be associated with protection against infection. Antibacterial antibody levels elicited with initial vaccination dropped progressively with the 2nd and 3rd vaccinations. Nevertheless, these antibodies did not seem to be necessary for protection against *A. pyogenes* conditions.

INTRODUCTION

Actinomyces pyogenes, formerly *Corynebacterium pyogenes* (Collins & Jones, 1982), is the cause of a wide variety of pyogenic infections in ruminants, pigs and, occasionally, horses (Smith, 1966). In cattle and sheep, pneumonia, mastitis, metritis, lymphadenitis, abscesses and wound infections occur (Addo & Dennis, 1977), endocarditis (Gillespie & Timoney, 1981) is seen, and the organism has been associated with abortions and infertility (Hinton, 1974; Addo & Dennis, 1979; Barth, Haase & Rehbock, 1976; Smith, Reynolds, Clark & Milbury, 1971). It has also been implicated as the cause of perinatal mortalities in lambs (Dennis & Bamford, 1966) and foot-abscess in sheep (Gardner, 1961).

The organism produces a soluble haemolytic toxin (Lovell, 1937) which has been used as a toxoid in vaccines to prevent summer mastitis in cattle (Brown & Stuart, 1943; Weitz, 1949; Lovell, Foggie & Pearson, 1950). Evaluation of the effect of *A. pyogenes* toxoid vaccines indicated that little or no protection was conferred (Packer, 1977), although the aluminium precipitated toxoid reduced the severity of clinical symptoms and the mortality which accompanies *A. pyogenes* mastitis (Brown & Stuart, 1943). Attempts to induce protection against pyogenic infection with bacterin-toxoid vaccines in mice failed to elicit solid immunity (Derbyshire & Matthews, 1963; Cameron, 1966). Immunizing mice with live organisms also failed to protect them from fatal infection (Durner & Werner, 1983). A bacterin-toxoid is produced by the VRI for use in cattle and sheep, annual usage being roughly a million doses. However, the efficacy of the vaccine in either cattle or sheep has not been evaluated. In this study, the immunity conferred by an *A. pyogenes* bacterin-toxoid was evaluated in sheep, using an intravenous challenge. Serology was performed to investigate the protective role of antitoxic and antibacterial antibodies.

MATERIALS AND METHODS

A. pyogenes isolate

A toxigenic *A. pyogenes* strain 23402, originally isolated from a case of bovine mastitis was used for the challenge, toxin production and preparation of antigen for the ELISA.

Challenge material

Strain 23402 was cultured on 200 ml of trypticase soy broth (TSB¹) to which 0.5 % of a 50 % glucose solution was added. The culture was incubated at 37 °C and shaken for 18 h at 100 rpm. The growth

was harvested by centrifugation and the cells were washed 3 times and resuspended in PBS so that each sheep received a total challenge dose of 5×10^8 organisms.

Serology

Antitoxin assay

The antitoxin content of the sheep sera was assayed, using the method of Capper (1953). Toxin was prepared by culturing 23402 on chopped meat medium overnight. This culture was then used as inoculum (5 % v/v) for the following growth medium: 10 g of proteose peptone (Difco² No. 2), 5 g of beef extract (Biolab), 5 g of NaCl, 2 g of yeast extract (Biolab) made up to 970 ml with distilled water (pH 7.3) and autoclaved for 30 min at 121 °C. This broth was supplemented with filtered 50 % glucose solution (10 ml) and horse serum (20 ml). The culture was incubated for 18 h at 37 °C on a shaker. When the pH dropped below 5.5, it was reset to 7.2 with 4 M NaOH, incubated for a further 2-3 h and the pH was finally adjusted to 7.3. The culture fluid was centrifuged and filtered through a 0.22 µm filter and titrated according to the method of Capper (1953) to determine the minimum haemolytic dose.

ELISA antigen preparation

The *A. pyogenes* isolate 23402 was cultured on 200 ml of TSB broth to which 0.5 % of a 50 % sterile glucose solution was added. The culture was shaken and incubated for 20 h at 37 °C. The cells were harvested by centrifugation, suspended in PBS containing 0.4 % formalin and incubated at 37 °C overnight. The cells were then made up in PBS to a density equivalent to McFarland standard No. 4. This suspension was then ultrasonicated for 6 min. The cell residues were removed by centrifugation and the supernatant fluid was then used as antigen. Linbro³ ELISA plates were coated using 200 ml of antigen per well, and incubated overnight at 37 °C.

Positive and negative serums

A positive serum was prepared by infecting a healthy 6-month old Dorper ewe intravenously with 10^8 *A. pyogenes* organisms, prepared as described under challenge material. The sheep was bled at 3-day intervals after infection and the sera tested against pre-infection serum from the same sheep, which was designated the negative serum. Serum drawn on the 15th day after infection was used as a positive serum.

¹ Biolab Chemicals, P.O. Box 14574, Verwoerdburg 0140

² Difco Laboratories, Detroit, Michigan

³ Flow Laboratories Ltd, P.O. Box 17, Second Avenue, Industrial Estate, Irvine Ayrshire Scotland

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TABLE 1 Response of vaccinated and control sheep to *Actinomyces pyogenes* challenge

Sheep No.	Status	Lesions on P.M.	ELISA index at time of challenge	Anti-haemolysin titre AU/ml at time of challenge
3223	Vaccinated	*Septicaemia, lung abscess	+	10 240
3146	Vaccinated	Endocarditis	-	2 560
3237	Vaccinated	No lesions	-	5 120
3175	Control	Endocarditis lung abscess	-	1 280
3163	Control	No lesions	-	0
3240	Control	No lesions	-	0

* = died 20 days post-challenge

ELISA method

The method used was modified from that of Worthington, Weddell & Penrose (1984). Test and standard sera were diluted to 1/100. Rabbit anti-sheep⁴ conjugate was used at a working dilution of 1:500. 0-phenylenediamine⁵ was stored and prepared as described by Worthington *et al.* (1984).

Positive reactions were recorded if the index (OD test serum)

was 2 or greater than 2.

(OD negative serum)

Experimental design

Six yearling Dorper ewes were held together in a separate camp on the grounds of the Veterinary Research Institute. Initially, all 6 sheep were bled and 3 were then vaccinated, using a bacterin-toxoid⁶ according to the manufacturers' recommendations, a course of 3 inoculations of 5 ml subcutaneously at 10 day intervals. Three sheep were held as controls. All 6 animals were bled at the time of the 2nd and 3rd inoculations, and then 3 weeks later before the sheep were challenged. The sheep were observed for clinical signs up to 3 weeks after challenge, when they were euthanized and post-mortem examinations were performed.

RESULTS

Clinical signs

For 3 days post-challenge all the ewes had intermittent fever reactions and showed limb stiffness. Joint swelling was observed in a few animals. After 3 days, the clinical signs subsided and the sheep appeared normal. There was no noticeable difference between the vaccinated and control groups on clinical evaluation. One of the vaccinated sheep died 20 days after challenge and was autopsied. The other 5 sheep were autopsied 30 days after challenge.

Pathology

The macroscopic findings are summarized in Table 1. Of the vaccinated group, Ewe 3223 died 20 days after challenge with septicaemia and lung abscessation. A purulent valvular endocarditis was noted in Ewe 3146, while no gross lesions were found in Ewe 3237. Of the control group, 2 animals were normal and 1 animal, 3175, had a purulent valvular endocarditis and lung abscessation.

Bacteriology

A. pyogenes was isolated from all pyogenic lesions, as well as from the organs of the septicaemic case. A summary of the post-mortem findings and

the serological status of the sheep at the time of challenge are shown in Table 1.

Serology

All 3 vaccinated ewes showed increasing antitoxin titres which rose after each inoculation. One control sheep (3175) initially had a low antitoxin titre, while the other 2 sheep had no antitoxin titres.

Initially, 2/3 of the vaccinated sheep were designated positive on ELISA results, but after the 2nd and 3rd inoculations the optical density indices dropped and only one sheep (3223) had a positive value at the time of challenge. Fig. 1 compares the antibacterial titre, as determined by ELISA, and the antihæmolysin titre of the control and vaccinated sheep before and during the experiment.

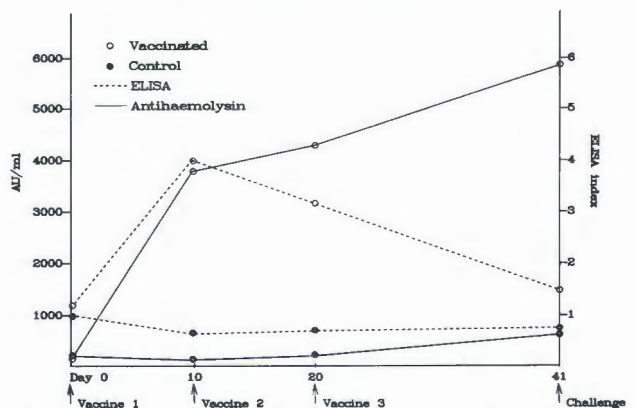


FIG. 1 Comparison of *A. pyogenes* antibacterial and antihæmolysin response in controls and vaccinated sheep

DISCUSSION

The recommended vaccination regime had the effect of raising substantially, the anti-hæmolysin titre and resulted in a 50-fold increase in the titre from the first inoculation to the time of challenge. However, high levels of antitoxin could not be associated with protection against pyogenic infection. The sheep with the highest antitoxin titre (3223) developed a septicaemia and died 20 days post-challenge.

Antibacterial antibody levels, as detected by the ELISA, rose after the initial vaccination, but fell progressively with subsequent inoculations. Despite this, 2/3's of the controls remained unaffected on challenge. It would appear from the above that protection against *A. pyogenes* infection may not be associated with either antitoxic or antibacterial antibodies. The poor performance of the vaccinated sheep, of which 2/3 developed pyogenic conditions compared to 1/3 of the controls, suggests that vaccination is not protective.

⁴ Dakopatts 42 Produktionvej, P.O. Box 1359, DK-2600, Glasstrup Denmark

⁵ Sigma Chemical Co., P.O. Box 14508, St Louis, Missouri 63178, USA

⁶ *Corynebacterium pyogenes* vaccine Reg. No. G135

Immunosuppression as a result of *A. pyogenes* immunization or infection has been postulated by some authors. Cameron (1966) noted that mice, immunised with *A. pyogenes* vaccine, appeared more susceptible to infection than control mice, although he later showed that mice could be protected against a low level of challenge (Cameron, Botha & Smit, 1976). Fekadu, Horn, Rantzien & Prage (1979), studying antibody levels in cattle with chronic *A. pyogenes* mastitis noted that the predominant immunoglobulin class, detected using ELISA, was IgM rather than the expected IgG response. This type of response has been associated with concurrent suppression of the cell-mediated response, caused by bacteria, such as *Mycoplasma bovis* (Bennett & Jasper, 1977). Other gram positive bacteria, such as *Listeria monocytogenes* (Otokunefor & Galsworthy, 1982) and *Corynebacterium parvum* (Scott, 1972), have been found to possess immunosuppressive as well as adjuvant properties.

The small number of animals and the artificial challenge route and dose used in this study made it difficult to draw specific conclusions. However, the results indicate that the *A. pyogenes* vaccine or the vaccination regimen adopted to enhance the production of antitoxic antibodies offers no protection against infection with the organism and may, in fact, be deleterious by possibly suppressing the host's cellular immunity.

This may be of particular significance in South Africa where the vaccine is widely used in conjunction with several live, attenuated, viral and other vaccines. It is therefore important that the immune response to *A. pyogenes* bacterin-toxoids should be investigated further.

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