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RESEARCH COMMUNICATION

A monovalent attenuated serotype 2 bluetongue virus vaccine confers homologous protection in sheep

P. HUNTER and J. MODUMO

Onderstepoort Biological Products Ltd, Private Bag X7, Onderstepoort, 0110 South Africa

ABSTRACT

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An outbreak of bluetongue caused by bluetongue virus serotype 2 virus in certain Mediterranean countries during 1999/2000, presented an opportunity to produce a monovalent type 2 vaccine. Since no data have been published previously on the protection conferred by the current live attenuated bluetongue vaccine strains used in the polyvalent vaccine, a challenge experiment was performed to determine the degree of homologous protection induced by the type 2 vaccine strain. The standard vaccine dose of 5 x 10^4 pfu of vaccine conferred 99.7 % protection against clinical disease and no viraemia was detected in the vaccinates.

Keywords: Attenuated, bluetongue vaccine, Mediterranean countries, monovalent, serotype 2

INTRODUCTION

Bluetongue is an arthropod-borne viral disease which affects mainly sheep. The causative bluetongue virus (BTV) belongs to the genus *Orbivirus* of the family *Reoviridae*. The disease is endemic in South Africa where 21 serotypes occur, of which 15 of these are considered to be pathogenic for sheep (Verwoerd & Erasmus 1994).

Because of the serious constraint which the disease presents for the sheep industry, an "attenuated" monotypic blood vaccine was produced and used for approximately 40 years with some success, but was withdrawn later due to safety and efficacy concerns (Theiler 1908). Once it was discovered that BTV can be grown in embryonated hen's eggs and that serial passage in this host causes attenuation of field strains, it was shown that these attenuated strains were safe and immunogenic (Howell 1969). When cell culture technology became available the egg-attenuated vaccine strains were plaque purified and cultured on cells; polyvalent vaccines produced from this material have been used with success for the last 50 years (Verwoerd & Erasmus 1994). However until the present no data have been published on the safety and efficacy of these strains.

The production of an attenuated monovalent BTV serotype 2 vaccine presented an opportunity to conduct an experimental challenge to assess homologous BTV 2 protection in sheep.

The attenuated BTV2 vaccine was produced using seed stock derived from the master seed of the Vryheid prototype strain which was originally attenuated in embryonated hen's eggs and plaque purified on cell culture. Virus culture was done using monolayers of BHK 21 Clone 13 cells. Virus and cell master seeds were tested for freedom from adventitious agents. The cells were cultured using Glasgow modification of Eagle's medium and donor bovine serum free from adventitious viruses. The BHK cells were grown in roller flasks and when the monolayers were confluent they were seeded using a 1/10 dilution of wet working stock prepared as for the vaccine. At maximal cytopathic effect the material was harvested,

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TABLE 1 Summary of results of challenge of vaccinates and controls with virulent BT 2 strain

Group	Antibody titre at time of challenge[a]	Post-challenge antibody titre (4 weeks)	Viraemia	Clinical index	% protection[d]
Vaccinates					99.7
3032	160	160	–	0.1	
3043	160	320	–	0.0	
3026	160	> 640	–	0.4	
3044	160	160	–	0.0	
				= 0.5	
Controls					0.0
3115	< 20	640	+[c]	19.5	
3090	< 20	ND[d]	+[c]	36.5	
				= 55.0	

[a] antibody titre expressed as reciprocal of last dilution to show 50% plaque reduction
[b] not done as sheep was euthanazed due to severity of BTV infection
[c] viraemia was recorded on days 3–7 post challenge
[d] percentage protection = 100—relative reaction (RR) where RR is CRI of test sheep as a percentage of the control (Huismans *et al.* 1987)

diluted with buffered lactose peptone and freeze-dried. Post freeze-drying titres of the harvest were determined on mouse fibroblast cells to be 2,3 x 10^5 pfu per mℓ. The standard minimum requirement for the vaccine is 5 x 10^4 pfu per dose.

A group of four BTV seronegative sheep which were held in insect-proof stables were vaccinated with one dose of vaccine. Another group comprised of two seronegative sheep constituted the controls; they were maintained under the same conditions as the vaccinates. The habitus and temperature of the vaccinated sheep were monitored daily. No clinical signs were seen. A clinical index calculated according to the method of Huismans *et al.* (1987) did not exceed the maximum permissible value of five for attenuated strains. The vaccinated sheep were bled 30 days post-vaccination and their sera tested together with the pre-vaccination sera using a plaque reduction test (see summary of results in Table 1). All vaccinates showed 50% neutralization in plaque reduction assays at a serum dilution of 1/160.

Challenge material was prepared by infecting a susceptible sheep with a virulent BTV2 field strain intravenously. The sheep was bled at maximal temperature reaction into the anticoagulant OCG (Erasmus 1990). The infected blood showed a titre of 10^5 pfu/mℓ on Vero cells.

The four vaccinated sheep plus the controls were challenged five weeks post-vaccination by intravenous injection of two ml of infected blood prepared previously in a susceptible sheep. All the challenged sheep were monitored daily for 14 days for clinical signs and bled daily into OCG for viraemia determinations. The sheep were scored according to the clinical index system (Huismans *et al.* 1987).

Both control sheep showed severe clinical signs which included the development of rectal temperature reactions of 42 °C, hyperaemia and erosions of the nasal mucosa, erosions in the mouth and hyperaemia of the coronary skin. They both became lame and one animal was subsequently euthanazed as it developed severe lung oedema which was unresponsive to treatment. On post mortem examination the latter showed evidence of multifocal necrosis of certain skeletal muscles. Apart from mild rectal temperature reactions which did not exceed 40.3 °C, none of the vaccinates developed any signs consistent with bluetongue.

Assays for vireamia in 6-day-old embryonated eggs showed that the controls were viraemic for five of the post-challenge days (from days 3–7). The vaccinates did not develop detectable viraemia. All surviving sheep were bled 30 days post-challenge to monitor seroconversion (Table 1).

Using the formulae previously described for calculating the protective index (Huismans *et al.* 1987), the vaccinates showed a protective index of 99.7% protection against challenge.

There are valid concerns about the safety of live attenuated bluetongue virus vaccines particularly concerning their teratogenicity when administered to ewes in the first half pregnancy (Young & Condy 1964). Transmission of vaccine strains by vectors was demonstrated in an experiment. However, these strains appeared to be poorly attenuated (Foster *et al.* 1968). High titres of circulating virus are required to infect insect vectors: Mellor (1990) calculated that titres of 10^4 insect infectious doses per mℓ of blood would be required to expose *Culicoides* vectors to one insect infectious dose.

Reassortment and reversion to virulence which are often cited as reasons for not using attenuated vaccines in non-endemic regions, have however not been demonstrated experimentally (Wark *et al.* 1982) or in the field. In the face of bluetongue outbreaks, even in non-endemic regions, the risk of reassortment would appear to be outweighed by the serious consequences of the disease to the sheep industry.

Although inactivated and recombinant bluetongue vaccines have been shown to be effective (Parker *et al.* 1975; Campbell 1985; Roy *et al.* 1990) these approaches present problems for vaccine production where multiple serotypes are involved. Under these conditions live attenuated BTV vaccines still present an effective and economical approach to the control of the disease.

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