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Short communication

An inactivated vaccine for the control of bluetongue virus serotype 16 infection in sheep in Italy

G. Savini *, G.F. Ronchi, A. Leone, A. Ciarelli, P. Migliaccio, P. Franchi, M.T. Mercante, A. Pini

Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise "G. Caporale", Via Campo Boario, 64100 Teramo, Italy

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Abstract

Because no suitable products are at the moment available to safely control the spread of BTV-16 in Europe, an inactivated vaccine was produced from the reference field isolate of bluetongue virus serotype 16. One group of six sheep was vaccinated subcutaneously with the inactivated vaccine twice, on days 0 and 28, whereas a second group of eight sheep was inoculated with saline solution and used as mock-vaccinated control animals. Seventy-eight days after the first vaccination, all sheep were inoculated subcutaneously with a suspension containing $10^{6.3}$ $TCID_{50}$ of a virulent reference BTV-16 isolate. Apart from a transient inflammatory reaction at the injection site, no adverse effects were reported following vaccination. All vaccinated animals developed high titres (7.3–9.3 $\log_2$(ED50%/50 μl)) of virus-specific neutralising antibodies and were resistant to challenge with BTV-16. Conversely, following challenge, control animals developed hyperthermia and long lasting high-titre viraemia.

Keywords: Bluetongue virus serotype 16; Inactivated vaccine; Sheep; Virus isolation; Virus neutralisation

1. Introduction

Bluetongue (BT) is an infectious, non-contagious disease of ruminants caused by an arthropod-borne virus belonging to the family *Reoviridae*, genus *Orbivirus*. The bluetongue virus (BTV) includes twenty-four distinct serotypes which are transmitted by haematophagous insects belonging to the family *Ceratopogonidae*, genus *Culicoides* (Mellor, 1992). Infection can cause severe clinical disease in sheep whereas, in other species including cattle and goats, the infection is mainly subclinical (Anderson et al., 1985). From 1998, BTV infection spread progressively all over the Mediterranean Basin, Balkan areas and more recently in Central Europe. Up till now, seven serotypes have been detected in the Mediterranean Basin: BTV-1, BTV-2, BTV-4, BTV-8, BTV-9, BTV-15 and BTV-16 (Mellor and Wittmann, 2002; OIE, 2006a,b,c,d,e,f,g,h). In Italy, the first evidence of BT infection was recorded in Sardinia on August 2000 and, since then, numerous

* Corresponding author at: Department of Virology, OIE Reference Laboratory for Bluetongue, Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise "G. Caporale", Via Campo Boario, 64100 Teramo, Italy. Tel.: +39 0861 332440; fax: +39 0861 332251.
E-mail address: g.savini@izs.it (G. Savini).

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outbreaks have been described involving BTV serotypes 1, 2, 4, 9 and 16. BT was later reported in Sicily, Calabria, Basilicata, Puglia, Campania, Lazio, Tuscany, Abruzzo, Molise, Umbria, Marche, Emilia Romagna and Liguria (Calistri et al., 2004; OIE, 2006f). In an attempt to reduce direct losses due to disease and indirect losses due to the trade embargo caused by virus circulation, the Italian government has, since February 2002, been carrying out a compulsory BT vaccination campaign of all domestic ruminants using modified-live virus vaccines (MLV) produced by Onderstepoort Biological Products (OBP), South Africa. Based on the serotype/s present in a given area, various MLV monovalent serotype formulations were used. During 2004, BTV-16 MLV was included in the vaccination campaign and many vaccinated and unvaccinated animals became ill as a result of BTV-16 vaccine strain infection (Monaco et al., 2006). The incidents were attributed to inadequate attenuation of the vaccine strain employed and the use of the monovalent MLV was discontinued a few months after the beginning of the campaign (Italian Ministry of Health, 2005). These events increased the concerns on safety issues regarding the MLV and prompted researchers to investigate new immune-stimulating products capable of preventing the dissemination of the virus. Inactivated vaccines for BTV serotypes 2 and 4 have recently been developed and successfully used during the 2005–2006 BTV vaccination campaigns.

Because no suitable products are available to safely control the spread of BTV-16, no strategies are currently applied for this serotype in Italy resulting in BTV-16 circulation. There is an urgent need for a safe and efficacious vaccine which could be used in the BTV vaccination campaign and that is capable of preventing the spread of BTV-16 in Italy. In this study a BTV-16 inactivated vaccine was developed from the BTV-16 reference serotype and its safety and efficacy was evaluated in sheep which is the species most susceptible to BTV.

2. Materials and methods

2.1. Cells and virus

Baby hamster kidney cells (BHK_{21}, C13) were maintained in minimal essential medium containing Earle's salts, L-glutamine and $NAHCO_3$ (EMEM) and supplemented with nystatin, colistin and neomycin sulphate, and 10% foetal calf serum (FCS). The BTV-16 reference strain, obtained from the Virology Department of the Onderstepoort Veterinary Institute, OIE Reference Laboratory for Bluetongue, Onderstepoort, South Africa, was used as seed virus.

2.2. Vaccine production and in process control

The seed virus was checked for the presence of contaminating bacteria, viruses, fungi and mycoplasmas (European Pharmacopoeia, 2001). Its identity was confirmed by serum neutralisation test. BHK_{21} confluent monolayers in 175 cm^2 culture flasks were used for virus amplification. When 80% of the monolayers showed CPE, the viral suspension was harvested, centrifuged at 203 × *g* for 10 min at 4 °C, the supernatant collected, dispensed in 1 ml aliquots and stored at −80 °C. The virus stock was checked for purity, infectivity titre and identity. For the preparation of a batch of experimental vaccine, BTV-16 at the third passage level was used to infect BHK_{21} monolayers in 850 cm^2 roller bottles. The viral suspension (VS) was aseptically collected and clarified by centrifugation at 9000 rpm (centrifuge Heraeus mod. Stratos 76000083) in continuous flow (rotor Heraeus Titanium 3049) and tested for purity, infectivity titre and protein concentration (BCATM Protein Assay Reagent Kit, Pierce, Rockford, IL). After passing the tests, the suspension was purified and 10-fold concentrated through a 300 kDa molecular cut cassette membrane. During the purification-concentration phase, the VS was kept on ice. The final product was again checked for purity (European Pharmacopoeia, 2001), virus identity and infectivity titre. For inactivation, Tris–hydroxymethyl-aminomethane (Tris) 1 M pH 8.0 was added to the VS and stirred for 10 min at room temperature. Beta-propiolactone diluted in PBS pH 7.4 was subsequently added to the VS to have a final dilution of the inactivant equal to 0.2% (v/v) (Parker et al., 1975). The suspension was stirred for 3 h at 37 °C and then for 18 h at 5 °C.

The inactivated VS was subsequently checked *in vitro* for inactivation as follows. Three 175 cm^2 tissue culture flasks containing confluent monolayers of BHK_{21} cells were each inoculated with 10 ml of inactivated VS. Three passages were carried out. If no CPE was observed at the end of the third passage, the