

Efficacy and safety studies on an inactivated vaccine against bluetongue virus serotype 2

B. Di Emidio^{†(1)}, P. Nicolussi⁽²⁾, C. Patta⁽²⁾, G.F. Ronchi⁽¹⁾, F. Monaco⁽¹⁾, G. Savini⁽¹⁾, A. Ciarelli⁽¹⁾ &
V. Caporale⁽¹⁾

(1) Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale', Via Campo Boario, 64100 Teramo, Italy
(2) Istituto Zooprofilattico Sperimentale della Sardegna 'G. Pegreff', Via Duca degli Abruzzi 8, 07100 Sassari, Italy

Summary

An inactivated vaccine was produced from an Italian field isolate of bluetongue virus serotype 2 (BTV-2) with a titre of $10^{7.8}$ TCID₅₀/ml. The virus was purified through a molecular cut cassette membrane, inactivated with β -propiolactone and emulsified with ISA 206 (Seppic) adjuvant. The vaccine was then tested for sterility, toxicity and safety in laboratory and target animals according to European Pharmacopoeia standards. Immunogenicity was assessed by inoculating subcutaneously 10 sheep and 10 goats each with 2 ml of the vaccine and 10 bovines each with 5 ml of the vaccine. A booster dose was inoculated after 14 days and no side-effects were reported following vaccination. Fourteen days after the booster dose, all vaccinated animals developed virus neutralising (VN) bluetongue (BT) antibody titres that on day 60 post vaccination ranged between 1/20 and 1/1 280. After one year, goats still had high VN antibody titres. Sheep were challenged 138 days after vaccination by subcutaneously inoculating 1 ml of $10^{5.6}$ TCID₅₀/ml of an Italian field isolate of BTV serotype 2; four unvaccinated animals were also inoculated and used as controls. Starting from day 6 post challenge, control animals developed a fever, with temperature ranging from 39.9°C to 40.6°C and lasting 48 h on average. BTV-2 was also isolated from the blood of control animals between days 4 and 20 post challenge. Conversely, neither fever nor viraemia were detected in the vaccinated animals that were challenged. A new trial with a larger number of animals, including all target species, has been planned and is in progress.

Keywords

Bluetongue – Cattle – Goat – Inactivated vaccine – Sheep – Vaccine – Virus – Virus neutralisation.

Introduction

Bluetongue (BT) is an infectious disease caused by a virus belonging to the family *Reoviridae*, genus *Orbivirus*; 24 serotypes with differing pathogenicity have been reported. The infection is transmitted by haematophagous insects belonging to the family *Ceratopogonidae*, genus *Culicoides*. The infection affects domestic and wild ruminants causing significant losses mainly in sheep and goats. Disease is rare in cattle and, when present, clinical signs are much milder. In those areas where the virus is present, losses in cattle are mainly due to the ban on animal transport; because of the effect on international trade, the economic repercussions of this disease in cattle can be severe. BT is included in

'List A' of the Office International des Épizooties (OIE) (10, 15, 16).

BT virus vaccines presently used in Europe are prepared with attenuated bluetongue virus (BTV) strains. These vaccines are usually safe and effective and are currently the most effective tool available to control BT outbreaks (1, 6). They stimulate good immune response and are quite inexpensive; however, the use of such vaccines in areas where the disease is considered exotic may give rise to difficulties with zoo-sanitary regulations and with the international trade of animals. Furthermore, they can produce undesirable side-effects in animals in poor health and are not recommended for use in ewes during the first three months of pregnancy, as they have been reported to cause abortions and foetal

malformations. Inactivated vaccines have been developed previously (9, 10, 11, 12); however, the safety and efficacy of these vaccines has been reported to be marginal. This report summarises the results achieved when an experimental inactivated vaccine against BTV-2, prepared according to European Pharmacopoeia guidelines, was administered to sheep, goats and cattle.

Materials and methods

Viral strain

The virus used in this study was BTV-2, strain 1486/A/00; the virus was obtained from the National Reference Centre for Exotic Diseases, Teramo, Italy (CESME: *Centro Studi Malattie Esotiche*). Before the strain was used for seed production, it was passaged three times in specific pathogen-free (SPF) embryonating chicken eggs inoculated intravenously, and then passaged three times in baby hamster kidney-21 (BHK-21) cell cultures. The virus was then plaque-purified according to the method described by Dulbecco (17) and the seed virus then lyophilised. The seed virus was checked for the presence of contaminating bacteria, viruses, fungi and mycoplasmas (8). Virus identity and titre were also confirmed.

Vaccine production and in-process control

The seed virus was passaged five times in cell culture and was harvested when the characteristic cytopathic effect (CPE) was fully developed. The viral suspension (VS) was aseptically harvested, purified through a molecular cut cassette membrane and inactivated with β -propiolactone at a final concentration of 0.2% (v/v) (14). Two aliquots of the inactivated VS were subsequently prepared and checked *in vitro* and *in vivo* for inactivation according to the protocol below.

In vitro inactivation test

Three 175 cm² tissue culture flasks containing confluent monolayers of BHK-21 cells were each inoculated with 10 ml of inactivated VS (first aliquot). After three passages, no viral CPE or BTV immunofluorescence was observed.

In vivo inactivation test

A second aliquot of VS was injected into four BT seronegative sheep (20 ml/sheep). Two further unvaccinated sheep were used as negative controls. Temperatures were recorded daily for 21 days and animals were observed for signs of abnormality. Furthermore, every second day, for 21 days, ethylene-diaminetetra-acetic acid (EDTA) blood

samples were collected and tested for the presence of BTV.

Once the inactivation was verified, the VS was emulsified with an equal volume of adjuvant Montanide[®] ISA 206. The vaccine was then tested in accordance with European Union (EU) legislation (5, 6, 7) and European Pharmacopoeia guidelines (8).

Safety test

The vaccine underwent safety tests by administering single and double doses to sheep, goats and cattle. The experiment was performed at the Centro Zootecnico e Caseario di Bonassai in Sassari, Italy, on unvaccinated and BT-seronegative animals.

Efficacy test

Vaccine efficacy tests were performed by screening the animals for neutralising antibodies and by monitoring them for clinical signs of BT. This was done on the same animals that had been used in the safety tests and which were inoculated with a single vaccine dose.

a) Antibody response

Plain blood samples were collected from sheep and goats to determine the neutralising antibody curve. Samples were collected from the sheep at the time (t) of vaccination (t=0) and 14, 60 and 137 days post vaccination (pv); samples were collected from goats at t=0, 14, 60, 137, 285 and 365 days pv. In this study, cattle were included only for the safety test; therefore, antibody levels in cattle were only determined at t=0, 14 and 60 days pv. No further testing was conducted on the cattle. Collected samples were tested using a competitive-enzyme linked immunosorbent assay (c-ELISA) (11) and the virus neutralisation (VN) test (13).

b) Challenge study

As sheep usually develop clinical signs following infection, they were chosen for the challenge studies. The experimental procedures were conducted according to current animal welfare provisions (2, 3, 4).

Vaccinated sheep and the respective control group, were infected with $10^{5.8}$ TCID₅₀ of BTV-2 137 days pv. Temperatures were recorded daily for 24 days and animals were observed for clinical signs. In addition, EDTA and plain blood samples were collected from each animal every second day for 24 days.

Results

Vaccine production and in-process control

Control of materials

Materials used during vaccine production fulfilled all the EU and European Pharmacopoeia guidelines (8); controls for sterility were also found to be satisfactory.

Primary seed virus and viral suspension

Identity and sterility were confirmed as well as the absence of viral contaminants. Following the purification process, the BTV titre of the VS was $10^{7.8}$ TCID₅₀/ml. Inactivation tests, verified both *in vitro* and *in vivo*, were satisfactory. No fever or side-effects were observed after injection of one or two doses. Virus was not isolated from the pv EDTA blood samples.

Tests on the final product

The final product was checked according to European Pharmacopoeia and EU provisions and was found to be free of contaminating bacteria, fungi and mycoplasmas.

Safety tests

Virus was not isolated from the blood samples collected from vaccinated animals. Temperatures were within the normal range. None of the animals in the trial developed any type of clinical signs. Only four animals, which were inoculated with a large dose of vaccine (10 ml/animal subcutaneously in the area of the neck), showed a slight phlogistic reaction at the inoculation site but this disappeared within 9 days.

Potency test

Following immunisation, all vaccinated animals developed virus neutralising and c-ELISA antibodies, confirming the vaccine to be immunogenic (Figs 1, 2 and 3). In goats, neutralising antibody levels remained constant throughout the observation period of more than one year (Fig. 2).

Challenge test

After challenge, vaccinated sheep did not show any clinical signs of BT disease and their temperatures remained normal; BT virus was not isolated from any of the challenged vaccinated animals. Control animals developed fever and clinical signs commencing on day 6 post challenge (Fig. 4). Viraemia was also detected from day 2 to day 20 post challenge (Fig. 5).

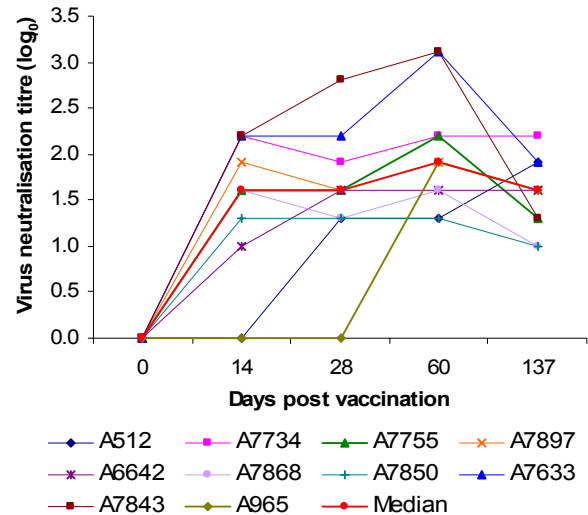


Figure 1
Neutralising antibody titres in sheep following vaccination with an inactivated bluetongue virus serotype 2 vaccine

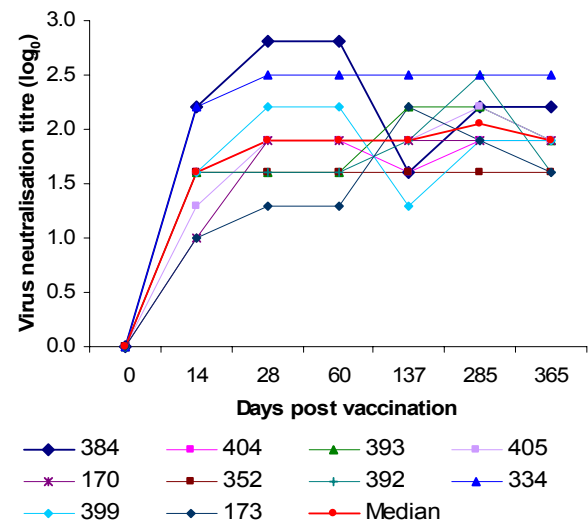


Figure 2
Neutralising antibody titres in goats following vaccination with an inactivated bluetongue virus serotype 2 vaccine

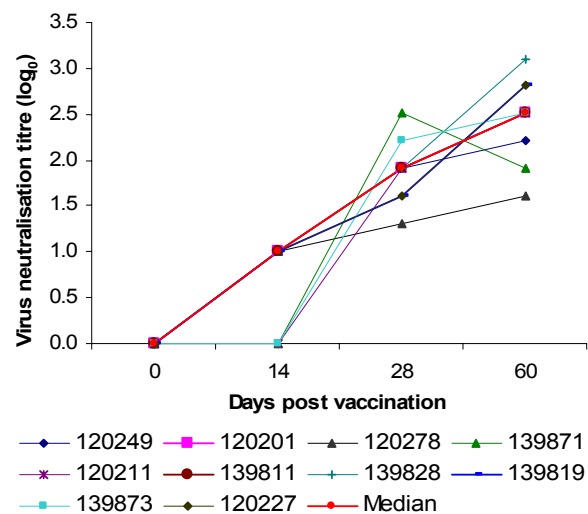


Figure 3
Neutralising antibody titres in cattle following vaccination with an inactivated bluetongue virus serotype 2 vaccine

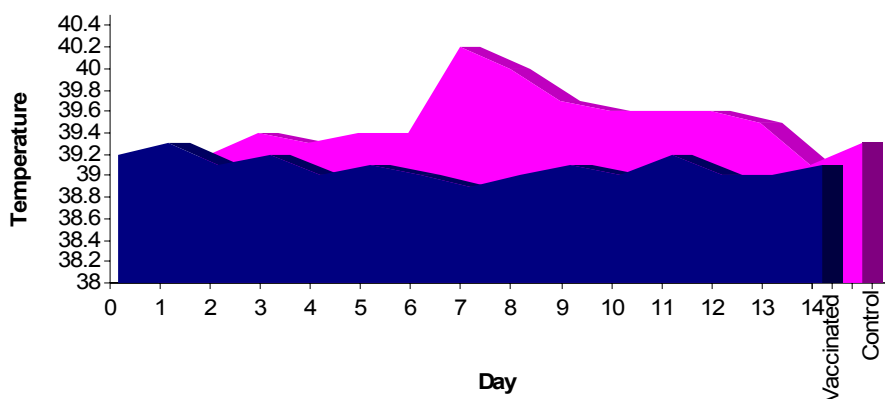


Figure 4
Temperatures in sheep vaccinated with an inactivated bluetongue virus serotype 2 vaccine after challenge with an homologous field isolate

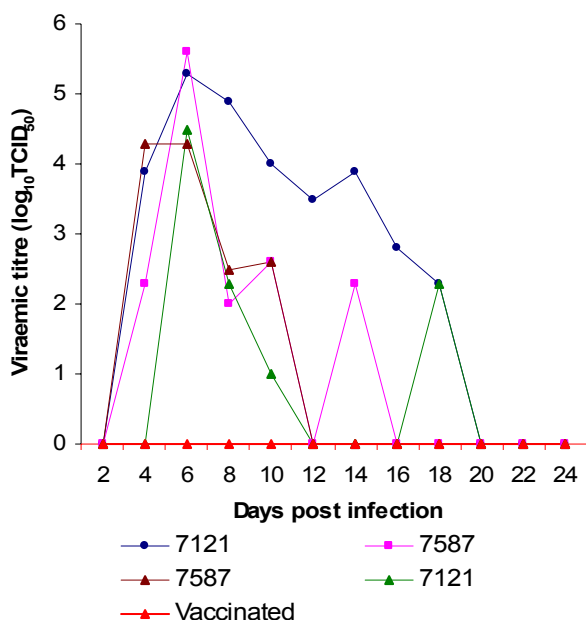


Figure 5
Viraemia in sheep vaccinated with an inactivated bluetongue virus serotype 2 vaccine after challenge with an homologous field isolate

Discussion and conclusions

The present study demonstrated that the experimental inactivated vaccine prepared from purified, inactivated and adjuvanted BTV-2 was safe and effective and met the requirements of the European Pharmacopoeia and the EU. Neither pyrexia nor other significant signs were observed in animals *pv*. The vaccine was safe and the capacity of β -propiolactone, at a final concentration of 0.2% (v/v), to inactivate BTV, was confirmed.

In all species tested, 100% seroconversion was observed following the first injection, and confirms

the findings of Murray *et al.* (12), Parker *et al.* (14) and Lefèvre and Desoutter (9). At 137 days, the animals that had been immunised with one or two doses of the vaccine were protected against challenge. Neutralising antibody titres in goats were still high after one year.

The results of this study have been compared with those from the Italian BTV vaccination campaign where an attenuated virus vaccine, produced by Onderstepoort Biological Products, was used.

Results of the vaccine comparison are as follows:

- the inactivated vaccine had no side-effects or limitations in its use in terms of safety
- all animals vaccinated with the inactivated vaccine developed neutralising antibodies, whereas only 92% of those vaccinated with the live-attenuated vaccine showed neutralising antibodies.

It would appear that the inactivated vaccine can be used in all epidemiological and production conditions requiring maximum security and effectiveness, including the need to discriminate between vaccinated and infected animals. To fulfil this final condition, a diagnostic kit that can distinguish between vaccinated and naturally infected animals is being developed. To test further these promising results and to evaluate the minimum protective vaccinal dose, a new trial with a larger number of animals, and including all target species, has been planned and is in progress.

References

- Agüero M., Arias M., Romero L.J., Zamora M.J. & Sánchez-Vizcaíno J.M. (2002). – Molecular differentiation between NS1 gene of a field strain bluetongue virus serotype 2 (BTV-2) and NS1 gene

- of an attenuated BTV-2 vaccine. *Vet. Microbiol.*, **86**, 337-341.
2. Erasmus B.J. (1975). – Bluetongue in sheep and goats. *Aust. Vet. J.*, **51**, 165-170.
 3. Erasmus B.J. (1975). – The epizootiology of bluetongue: the African situation. *Aust. Vet. J.*, **51**, 196-198.
 4. Erasmus B.J. (1980). – The epidemiology and control of bluetongue in South Africa. *Bull. Off. Int. Épiż.*, **92**, 461-467.
 5. European Commission (1991). – Commission Directive 91/412/EEC of 23 July 1991 laying down the principles and guidelines of good manufacturing practice for veterinary medicinal products. *Off. J.*, **L 228**, 17/08/1991, 70-73.
 6. European Commission (2001). – Commission Decision 2001/141/EC of 20 February 2001 for the implementation of a bluetongue vaccination programme in certain parts of the protection zone in Italy and the purchase by the Community of vaccine for this purpose (notified under document number C(2001) 424). *Off. J.*, **L 050**, 21/02/2001, 23-24.
 7. European Council (1981). – Council Directive 81/852/EEC on the approximation of the laws of the Member States relating to analytical, pharmacotoxicological and clinical standards and protocols in respect of the testing of veterinary medicinal products. *Off. J.*, **L 317**, 06/11/1981, 53-98.
 8. European Pharmacopoeia (2001). – Addendum No. 0016. Council of Europe, Strasbourg, 1657-1661.
 9. Lefèvre P.-C. & Desoutter D. (1988). – La fièvre catarrhale du mouton (bluetongue). Institut d'élevage et de médecine vétérinaire des pays tropicaux (IEMVT), Études et synthèses, Montpellier, 117 pp.
 10. Lelli R., Calistri P., Rolesu S., Patta C., Sulis F. & Ulleri R. (2000). – Bluetongue: un problema anche italiano. *Praxis Vet.*, **21**, 2-7.
 11. Lelli R., Langella V., Portanti O., Luciani M., Di Emidio B. & Conte A.M. (2003). – Production of a competitive ELISA kit for the serological diagnosis of bluetongue disease. *Vet. Ital.*, **47**, 5-13.
 12. Murray P.K. & Eaton B.T. (1996). – Vaccine for bluetongue. *Aust. Vet. J.*, **73**, 207-210.
 13. Office International des Épiżooties (OIE) (2000). – Bluetongue, Chapter 2.1.9. *In* Manual of standards of diagnostic tests and vaccines. OIE, Paris, 153-167.
 14. Parker J., Hernimann K.A.J. & Gibbs E.P.J. (1975). – An experimental inactivated vaccine against bluetongue. *Vet. Rec.*, **96**, 284-287.
 15. Pini A. (1976). – A study on the pathogenesis of bluetongue: replication of the virus in the organs of infected sheep. *Onderstepoort J. Vet. Res.*, **43**, 159-164.
 16. Pini A., Coackley W. & Ohder H. (1966). – Concentration of bluetongue virus in experimentally infected sheep and virus identification by immunofluorescence technique. *Archiv Ges. Virusfor.*, **18**, 385-390.
 17. Poli G. & Cocilovo A. (1996). – Microbiologia e immunologia veterinaria. UTET, Turin, 421- 422.