Virological and serological response of cattle following field vaccination with bivalent modified-live vaccine against bluetongue virus serotypes 2 and 9

F. Monaco[(1)], N. De Luca[(2)], P. Spina[(2)], D.Morelli[(1)], I. Liberatore[(1)], R. Citarella[(1)], A. Conte[(1)] & G. Savini[(1)]

(1) Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale', via Campo Boario, 64100 Teramo, Italy
(2) Azienda Sanitaria Locale di Pescara, via R. Paolini, 65100 Pescara, Italy

Summary

Following the bluetongue (BT) epidemic in Italy, the government initiated a vaccination campaign involving all domestic ruminants (cattle, sheep and goats) in the affected and adjacent areas to create a resistant population and to reduce virus circulation. Based on the serotypes circulating in the affected areas, monovalent BT virus (BTV) serotype 2 (BTV-2), or bivalent BTV-2 and BTV-9, modified-live vaccines were used. These are manufactured by Onderstepoort Biological Products in South Africa and, because they are recommended for use in sheep only, very little data exists on their use in cattle under field conditions. To evaluate duration and levels of viraemia and the antibody response following vaccination, 30 cattle in various stages of pregnancy were selected and vaccinated with a bivalent BTV-2/BTV-9 vaccine. Blood samples were taken from the animals three times a week for two months and screened using the competitive enzyme-linked immunosorbent assay (c-ELISA) and the virus neutralisation (VN) test. Intravenous egg inoculation, followed by two blind passages in Vero cells, was used to isolate BTV-2 and BTV-9 from ethylene-diaminetetra-acetic acid (EDTA) blood samples, and virus titres determined in viraemic animals. Titres against BTV were detected in 27 animals between days 4 and 35 post vaccination (pv). Viraemia peaked on day 9 pv with average viral titres of $10^{4.5}$TCID$_{50}$/ml. From day 9 pv, the c-ELISA detected antibodies in all animals while low VN titres were observed commencing on day 18 pv. Furthermore, VN antibody to BTV-2 was detected in only 17 of the animals vaccinated and to BTV-9 in 27 animals.

Keywords

Bivalent vaccine – Bluetongue – Cattle – Competitive enzyme-linked immunosorbent assay – Italy – Viraemia – Virus – Virus neutralisation.

Introduction

Bluetongue (BT) virus (BTV) is the etiological agent of BT, a non-contagious, arthropod-borne disease of both domestic and wild ruminants. Throughout tropical and temperate regions of the world (3, 5, 8) its distribution coincides with the presence of competent insect vectors, haematophagous biting midges of the genus *Culicoides* (3, 15). BT can produce an acute disease in sheep with a mortality rate reaching 10% or more, incurring major production losses. However, in cattle, the infection is typically asymptomatic (2, 5) and is characterised by viraemia lasting 30 to 45 days (5, 6, 9, 10, 14). Due to its ability to spread rapidly under suitable circumstances, BT is classified a 'List A' disease by the Office International des Épizooties (OIE). This means that in infected areas, trade bans will be imposed on the susceptible species, often with serious socio-economic consequences. Vaccination is one of the most effective preventive tools against viral diseases and especially for those transmitted by insects. Since 2000, Italy has experienced the most severe outbreaks of BT ever to be recorded. In an

attempt to reduce direct losses due to disease and indirect losses due to virus circulation, the Italian government has implemented a compulsory BTV vaccination campaign involving all susceptible domestic ruminants since May 2001. Based on the serotype/s present in a given area, a monovalent BTV-2 or a bivalent BTV-2/BTV-9 modified-live vaccine, produced by Onderstepoort Biological Products (OBP) in South Africa, was used. The vaccine was produced by the serial passage of virulent field strains of BTV in embryonating chicken eggs and in cell cultures. This vaccine is recommended for use in sheep only. The purpose of this study was to evaluate duration and levels of viraemia and antibody kinetics in cattle after immunisation with the bivalent modified-live BTV-2/BTV-9 vaccine to determine whether vaccinated cattle serve as a source of BT vaccine virus to bloodsucking arthropods.

Materials and methods

Sixty cattle at various stages of pregnancy were selected, 30 of which were vaccinated with the bivalent BTV-2/BTV-9 vaccine. All animals tested negative against the most common reproductive diseases (bovine virus diarrhoea, infectious bovine rhinotracheitis, bovine herpesvirus-4, salmonellosis, chlamydiosis, neosporosis and brucellosis). Antibodies to BT were not detected in their blood prior to vaccination. Each vaccine serotype was tested for contamination and titrated on Vero cells. Animals were inoculated with 1 ml of vaccine in the region of the neck.

Vaccinated animals and negative controls were kept under field conditions and stabled on six different farms. All animals were checked daily for clinical signs and body temperatures were recorded daily for 15 days post vaccination (pv). Ethylene-diaminetetra-acetic acid (EDTA) and plain blood samples were collected from each animal three times a week for 30 days and then twice a week for the following month. EDTA blood samples were screened for the presence of BTV and if virus was detected, the virus titre was determined for each of the serotypes isolated as described previously (13). The competitive enzyme-linked immunosorbent assay (c-ELISA) (7) and the virus neutralisation (VN) test (4) were used to detect the presence of BTV antibodies. Intravenous egg inoculation followed by two blind passages in Vero cells was used to isolate BTV from EDTA blood samples according to the method described by Savini *et al.* (12). The duration of viraemia was analysed using the Kaplan-Meier Survival technique (Spss® 11.0.1) in which the event is the absence of detectable BTV-2 and/or BTV-9 viraemia. The survival probability at each time interval (i.e. the probability of an animal being viraemic at time t) was interpolated through the linear regression function in Spss® 11.0.1, and the date on which the probability of being viraemic decreases below 0.01, was calculated.

Results

No viral or bacterial contaminants were detected in the vaccine. A dose of vaccine contained $10^{4.37}$TCID$_{50}$/ml BTV-2 and $10^{4.24}$TCID$_{50}$/ml BTV-9. The vaccinated animals did not show any clinical signs of BT infection. The calves born from the vaccinated cows during the experiment were healthy and no abortions or reproductive diseases were observed.

BTV-2 titres were detected in the circulating blood of 22 (73.3%) vaccinated animals between day 8 and day 28 pv. Viraemia peaked on day 11 pv with average viral titres of $10^{3.8}$TCID$_{50}$/ml. BTV-9 caused detectable viraemia in 25 (83.3%) cattle commencing on day 4 pv and lasting 24 days; maximum viral titre was detected on day 9 pv with an average titre of $10^{4.7}$TCID$_{50}$/ml (Fig. 1). None of the negative controls developed detectable viraemia. Commencing on day 9 pv, c-ELISA antibodies were detected in all vaccinated animals while low VN titres were observed, which commenced on day 18 pv.

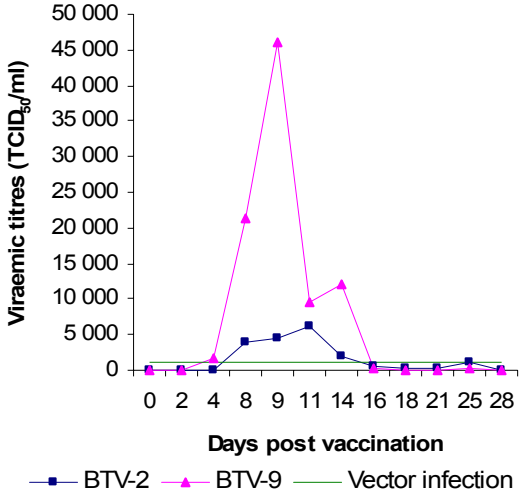

Figure 1
Bluetongue virus titres in cattle following vaccination with a bivalent BTV-2/BTV-9 modified-live vaccine

Antibodies against BTV-2 were detected in 29 animals commencing on day 16 pv with the highest titre on day 42 pv. All animals except one developed an immune response to BTV-9; VN antibody was detected on day 18 pv, two days later than BTV-2, and BTV-9 antibody titre peaked on

day 35 pv (Fig. 2). None of the control animals developed antibody against BTV-2 or BTV-9. Figure 3 gives the interpolation of cumulative probability of viraemic animals after X days. The linear equation was $y = 1.3036 - 0.0404x$. The probability of an animal being viraemic after 32 days is less than 0.99.

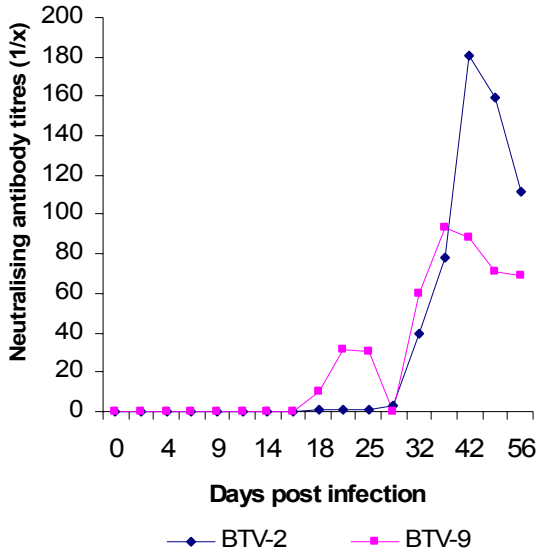

Figure 2
Mean neutralising antibody titres in cattle following vaccination with a bivalent BTV-2/BTV-9 modified-live vaccine

Discussion

Following the incursion of BT into Italy, the vaccination of all domestic ruminants (cattle, sheep and goats) was implemented in affected and in adjacent areas to create a resistant population and to reduce virus circulation. Based on the serotype/s circulating in the affected areas, a monovalent BTV-2 or bivalent BTV-2/BTV-9 modified-live vaccine was used. These vaccines are manufactured by OBP in South Africa and, because they are recommended for use in sheep only, no data exists on the vaccination of cattle under field conditions. Modified-live vaccine virus has to replicate in order to stimulate a protective immunity; in the process, they may induce a mild or subclinical illness in the immunised hosts. In this study, the vaccine did not induce any clinical signs of the disease in the vaccinated animals.

One of the potential drawbacks which might occur following immunisation with a modified-live vaccine is its subsequent potential transmission by insects. Consequently, it is crucial to determine the duration of viraemia and the peak vaccine virus titre attained. Previous studies have demonstrated that, in cattle, BTV persists in the erythrocytes, allowing the virus to remain in the blood despite the presence of neutralising antibodies (1, 2, 6, 14). In this study,

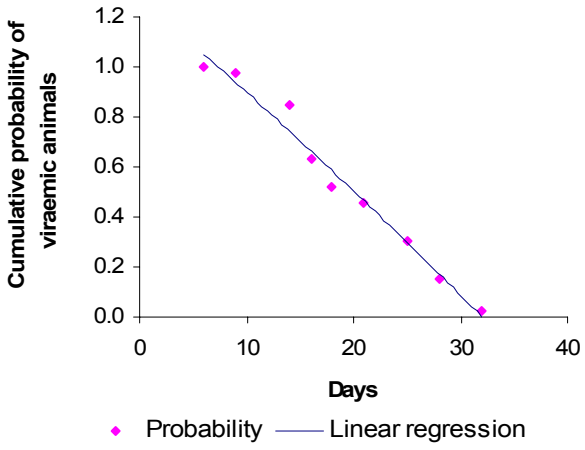

Figure 3
Interpolation of cumulative probability of animals being viraemic after X days following vaccination with a bivalent BTV-2/BTV-9 modified-live vaccine

viraemia persisted for 28 days and neutralising antibodies and virus were present from days 18 to 28 pv. It is critically important to understand whether the levels of vaccine viral titres in vaccinated cattle are high enough to infect locally active *Culicoides* because in this way the virus can be maintained in the environment. In this study, both BTV-2 and BTV-9 virus titres exceeded the value of 10^3TCID$_{50}$/ml for 6 and 12 days respectively; this titre is considered the threshold for possible *Culicoides* infection (11) (Fig. 1). Based on this hypothesis, the Kaplan-Meier survival technique was applied to assess the risk of moving viraemic animals after immunisation. According to this formula, cattle could be moved safely 32 days following immunisation with bivalent BTV-2/BTV-9 modified-live vaccine. After this time lapse, the risk of spreading vaccine viruses through bloodsucking insects is less then 0.01%.

Even though the bivalent vaccine used in this study was capable of inducing an immune response in almost all the vaccinated animals, seroconversion was not correlated with the titre of virus in the blood. In fact, although BTV-9 replication induced viraemic titres ($10^{4.7}$TCID$_{50}$/ml) higher than BTV-2 ($10^{3.8}$TCID$_{50}$/ml), the average BTV-9 neutralising antibody titres were lower than those observed for BTV-2.

References

1. Brewer A.W. & MacLachlan N.J. (1992). – Ultrastructural characterization of the interaction of

bluetongue virus with bovine erytrocytes *in vitro*. *Vet. Pathol.*, **29**, 356-359.

2. Brewer A.W. & MacLachlan N.J. (1994). – The pathogenesis of bluetongue virus infection of bovine blood cells *in vitro*: ultrastructural characterization. *Arch. Virol.*, **136**, 287-298.
3. Erasmus B.J. (1975). – Bluetongue in sheep and goats. *Aust. Vet. J.*, **51**, 165-170.
4. Gard G.P. & Kirkland P.D. (1993). – Bluetongue virology and serology. *In* Australian standard diagnostic techniques for animal diseases (L.A. Corner & T.J. Bagust, eds). CSIRO Information Services, Melbourne, 1-17.
5. Gibbs E.P. & Greiner E.C. (1994). – The epidemiology of bluetongue. *Comp. Immunol. Microbiol. Infect. Dis.*, **17**, 207-220.
6. Koumbati M., Mangana O., Noumikou K., Mellor P.S. & Papadopoulos O. (1999). – Duration of bluetongue viraemia and serological responses in experimentally infected European breeds of sheep and goats. *Vet. Microbiol.*, **64**, 277-285
7. Lelli R., Portanti O., Langella V., Luciani M., Di Emidio B. & Conte A. (2003). – Produzione di un kit ELISA competitiva per la diagnosi sierologica della bluetongue. *Vet. Ital.*, **39** (47), 5-13
8. MacLachlan N.J. (1994). – The pathogenesis and immunology of bluetongue virus infection of ruminants. *Comp. Immunol. Microbiol. Infect. Dis.*, **17**, 197-206.
9. MacLachlan N.J., Heidner H.W. & Fuller F.J. (1987). Humoral immune response of calves to bluetongue virus infection. *Am. J. Vet. Res.*, **48**, 1031-1035.
10. MacLachlan N.J., Rossitto P.V., Jagels G., Moore F.P. & Heidner H.W. (1990). – The pathogenesis of experimental bluetongue virus infection of calves. *Vet. Pathol.*, **27**, 223-229.
11. Murray P.K. & Eaton B.T. (1996). – Vaccine for bluetongue. *Aust. Vet. J.*, **73**, 207-210.
12. Savini G., Goffredo M., Monaco F., Di Gennaro A., Cafiero M.A., Baldi L., De Santis P., Meiswinkel R. & Caporale V. (2004). – Bluetongue virus (BTV) isolations from the Obsoletus Complex (*Culicoides*, Diptera: Ceratopogonidae) in Italy. *Vet. Rec.* (in press).
13. Savini G., Monaco F., Conte A., Migliaccio P., Casaccia C., Salucci S. & Di Ventura M. (2004). – Virological and serological response of sheep following field vaccination with bivalent modified-live vaccine against bluetongue virus serotypes 2 and 9. *In* Bluetongue, Part II (N.J. MacLachlan & J.E. Pearson, eds). Proc. Third International Symposium, Taormina, 26-29 October 2003. *Vet. Ital.*, **40** (4), 631-634.
14. Singer R.S., MacLachlan N.J. & Carpenter T.E. (2001). – Maximal predicted duration of viraemia in bluetongue virus-infected cattle. *J. Vet. Diag. Invest.*, **13**, 43-44.
15. Tabachnick W.J. (1996). – *C. variipennis* and bluetongue virus epidemiology in United States. *Ann. Rev. Entomol.*, **41**, 23-43.