Serological response in cattle and sheep following infection or vaccination with bluetongue virus

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Summary

Data from various experimental and field studies were compiled and analysed to evaluate the serological response in sheep and cattle against different bluetongue (BT) virus (BTV) vaccine combinations (Onderstepoort Biological Products, South Africa); the accuracy of diagnostic procedures commonly used for detecting BTV antibodies was also assessed. Using the competitive enzyme-linked immunosorbent assay (c-ELISA) (IZSA&M, Teramo, Italy) and the virus neutralisation (VN) test, antibody responses were evaluated under the following vaccination regimes: monovalent modified-live vaccine against BTV-2 in cattle and sheep, monovalent modified-live vaccine against BTV-9 in sheep, and bivalent modified-live vaccine against BTV-2 and BTV-9 in cattle and sheep. The data were compared to serological results observed in cattle and sheep infected with Italian field strains of BTV-2 or BTV-9. The c-ELISA consistently detected antibodies earlier than the VN test in both livestock species and against all BTV serotypes. The highest and most rapid antibody responses were observed in sheep infected in the field. In cattle and in sheep, high VN titres were detected using monovalent vaccines, while bivalent vaccines initiated lower antibody titres that developed more slowly.

Keywords

Bluetongue – Cattle – Competitive enzyme-linked immunosorbent assay – Italy – Sheep – Vaccine – Viraemia – Virus – Virus neutralisation.

Introduction

The outbreak of bluetongue (BT) and subsequent vaccination using monovalent and bivalent modified-live vaccines have resulted in the widespread immunisation of domestic ruminants across Italy. In the first three seasons of the outbreak, animals were exposed to bluetongue virus (BTV) serotype 2 (BTV-2) and/or BTV-9 field strains, monovalent BTV-2 modified-live vaccine and bivalent BTV-2 and BTV-9 modified-live vaccine. Various immunisation scenarios were studied under controlled conditions and serological responses, following immunisation with different BTV vaccine combinations and following infection with field strains, were evaluated. These data were also used to assess the accuracy of diagnostic procedures commonly used to detect BTV antibodies.

Materials and methods

The BTV-2 and BTV-9 monovalent modified-live vaccines used in this study were produced by Onderstepoort Biological Products in South Africa. A single dose of the monovalent modified-live vaccine against BTV-2 contained $10^{5.15}$TCID$_{50}$/ml of virus, while that of the BTV-9 monovalent vaccine contained $10^{5.8}$TCID$_{50}$/ml of virus. A single dose of the combined BTV-2/BTV-9 monovalent modified-live vaccine contained $10^{4.37}$TCID$_{50}$/ml of BTV-2 and $10^{4.24}$TCID$_{50}$/ml of BTV-9. BTV-2 challenge material was prepared from the spleen of a naturally infected sheep that died during the 2000 BT outbreak in Sardinia; the BTV-9 challenge material was prepared from blood sampled from a naturally infected cow in Calabria as described by Savini *et al.* (5).

To study the serological responses to field isolates of BTV-2 and BTV-9 in sheep, two groups of five ewes each were randomly selected; one group was challenged by subcutaneous injection of 1 ml of $10^{5.8}$TCID$_{50}$ of BTV-2 and the other group challenged with $10^{5.8}$TCID$_{50}$ of BTV-9. The same dose of the BTV-2 field isolate was used to subcutaneously infect four cattle. All challenged animals were kept in an insect-free stable for the duration of the study. As described previously, a further 44 sheep and 30 cows were selected (3, 6) and the bivalent BTV-2/BTV-9 modified-live vaccine administered subcutaneously. Also, monovalent BTV-2 modified-live vaccine was administered subcutaneously to seven sheep and five cows, whilst monovalent BTV-9 modified-live vaccine was administered to five sheep. Serum samples were tested for the presence of BTV antibodies using a competitive enzyme-linked immunosorbent assay (c-ELISA) (2) and the virus neutralisation (VN) test (1). Positive and negative controls for the VN were kindly provided by the OIE reference laboratory of the Onderstepoort Veterinary Institute (OVI) in South Africa.

Results

Apart from four sheep inoculated with the bivalent vaccine, all the remaining animals (96.2%) developed c-ELISA and virus neutralising antibody titres. Table I indicates the date at which antibody was first detected using the BT c-ELISA and the date at which all animals developed BTV c-ELISA antibodies. In general, higher neutralising antibody titres were observed after animals had been infected with field isolates of the two virus serotypes.

Table I
Days on which cattle and sheep showed c-ELISA antibodies following vaccination or experimental infection with bluetongue virus serotypes 2 and 9

BTV serotypes	Species	Days following vaccination or infection	
		First positive animals	100% positive animals
BTV-2 field isolate	Bovine	14	14
	Ovine	7	7
BTV-9 field isolate	Bovine	Not done	Not done
	Ovine	11	11
BTV-2 modified-live vaccine	Bovine	10	10
	Ovine	14	21
BTV-9 modified-live vaccine	Bovine	Not done	Not done
	Ovine	9	9
BTV-2 and BTV-9 bivalent modified-live vaccine	Bovine	9	19
	Ovine	6	16

However, in both species VN titres were also obtained after immunisation using a monovalent vaccine while animals vaccinated using a bivalent vaccine developed antibody later and had lower antibody titres (Figs 1, 2, 3 and 4). The most rapid response, with the highest titres, occurred in sheep infected with field isolates of BTV (Fig. 4).

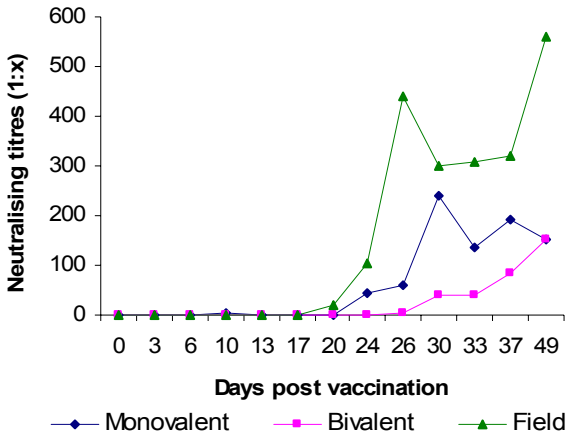

Figure 1
Bluetongue virus (BTV) serotype 2 (BTV-2) neutralising titres in cattle following immunisation using a monovalent BTV-2 modified-live vaccine, a bivalent BTV-2/BTV-9 vaccine or following experimental infection with a BTV-2 field isolate

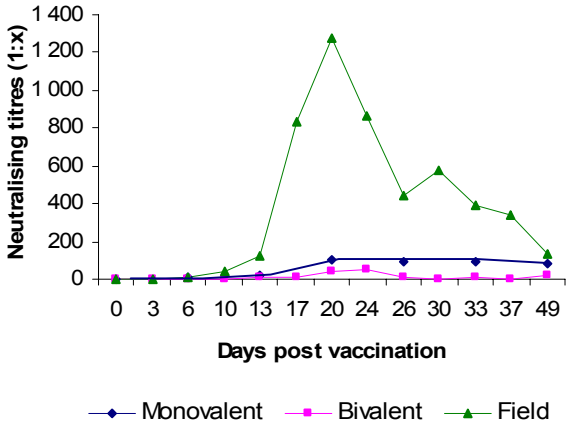

Figure 2
Bluetongue virus (BTV) serotype 2 (BTV-2) neutralising titres in sheep following immunisation using a monovalent BTV-2 modified-live vaccine, a bivalent BTV-2/BTV-9 vaccine or following experimental infection with a BTV-2 field isolate

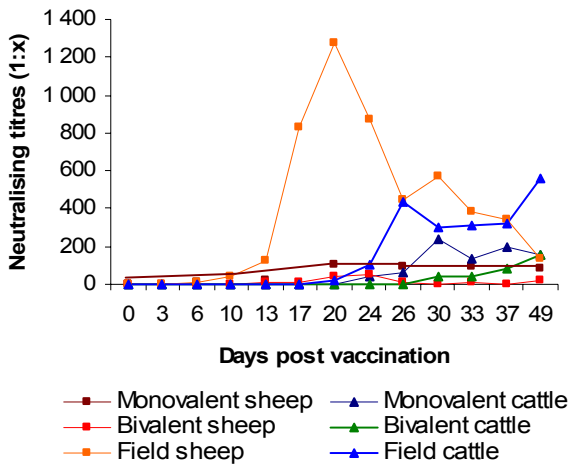

Figure 3
Bluetongue virus (BTV) serotype 2 (BTV-2) neutralising titres in cattle and sheep following immunisation using a monovalent BTV-2 modified-live vaccine, a bivalent BTV-2/BTV-9 vaccine or following experimental infection with a BTV-2 field isolate

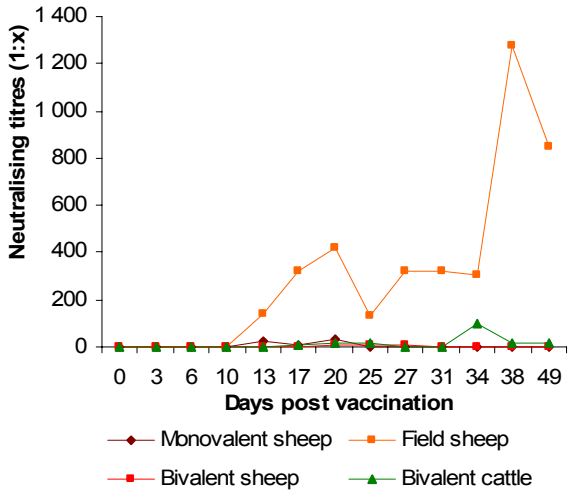

Figure 4
Bluetongue virus (BTV) serotype 9 (BTV-9) neutralising titres in cattle and sheep following immunisation using a monovalent BTV-9 modified-live vaccine, a bivalent BTV-2/BTV-9 vaccine or following experimental infection with a BTV-9 field isolate

Discussion and conclusions

One of the principal measures used to control the 2000-2003 outbreaks of BTV in Italy was the vaccination of all susceptible ruminants using monovalent BTV-2 and bivalent BTV-2 and BTV-9 modified-live vaccines. The evaluation of the immune responses generated by these vaccines provided useful information not only on the probable immune status of the animals after vaccination but also on the value of the diagnostic tests. As observed previously (4), the c-ELISA consistently detected antibodies earlier than the VN test and against both serotypes of BTV. As expected, the field isolates of BTV-2 and BTV-9 elicited higher VN titres than the modified-live vaccine strains. Although this discrepancy was evident for both serotypes in sheep, it was substantially greater for BTV-9, suggesting that the South African vaccine against this strain had a reduced immunogenic capacity.

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