Bluetongue vaccination in Europe: the Italian experience

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Summary

The incursion of bluetongue (BT) into Italy in August 2000 caused heavy economic losses, partly due to the disease itself, but mostly because of disruption caused to the national animal trade structure. To limit direct losses and the circulation of BT virus (BTV), the Italian Ministry of Health ordered, on 11 May 2001, the vaccination of all susceptible domestic ruminant species (i.e. sheep, goats, cattle and water buffalo) in both infected and surrounding areas. The vaccination strategy was based on a risk assessment that suggested it would prevent direct economic losses and significantly reduce virus circulation. Vaccination of the target animal populations commenced in January 2002, prior to the epidemic peak of BT that began in July 2002. The proportion of vaccinated animals differed between the various regions and the varying levels of vaccination of these populations had clear consequences on the occurrence of clinical disease and the spread of BTV infection. In those regions where more than 80% of the target population were properly vaccinated, the disease disappeared almost completely and virus circulation was reduced significantly. The importance of this reduced circulation of BTV (i.e. infection did not spread from affected areas) was immediately obvious in areas affected by the less virulent BTV serotype 9 where, despite the virtual absence of clinical disease, trade of animals to other areas was prohibited. The areas affected by the highly virulent BTV-2 also benefited from vaccination because it eliminated clinical disease while animal movements were prohibited. The main consequence of the reduction of virus circulation after vaccination, as documented by serological surveillance, was a significantly reduced expansion of the areas that were subjected to animal movement restrictions. Subsequently, analysis of surveillance data, coupled with specific risk assessments, led to a progressive relaxation of movement restrictions even in areas where the infection was still present but where most of the population had been adequately vaccinated.

The effectiveness of the strategy used in Italy (i.e. vaccination of all domestic ruminants) was reinforced by extensive experimental and field studies. The aim of these studies was to:

a) evaluate levels of individual and herd immunity and resistance to challenge conferred by vaccination, and

b) quantify the frequency and severity of the adverse effects of vaccination on domestic ruminants.

Ongoing research has focused on the ability of vaccination to suppress or reduce viraemia in ruminants following natural challenge by a virulent BTV strain. These studies address the issue of safety of the trade and movement of vaccinated animals that originate from areas in which BTV continues to circulate and could justify the reversal in current policy that restricts the international trade of animals vaccinated against BT.

Keywords

Bluetongue– Cattle – Italy – Sheep – Vaccine – Vaccination.

In November 2000, after the incursion of bluetongue into Sardinia on 18 August (10), the Balearic islands on 29 September (2) and Corsica on 18 October (1), the European Union issued Directive 2000/75/CE (9) that fixed specific rules for the control and the eradication of BT. Specifically, the Directive stipulated the following:

a) demarcation of a protection zone with a radius of 100 km around outbreaks of BT or around any farm on which virus circulation was confirmed
b) establishment of a surveillance zone that extended 50 km around the protection zone
c) slaughter of animals to prevent the spread of the epidemic and to destroy, eliminate, burn and bury their carcasses
d) implementation of serological and entomological surveillance programmes in the protection and surveillance zones
e) ban on animal movements from protection to surveillance zones.

To complement these measures, the Directive foresaw the possibility of conducting a vaccination programme in the protection zone but, since the Directive contained no specific criteria on serological and entomological surveillance, each country could freely propose its own programme to the European Commission, taking into account specific national needs and geographical or livestock breeding conditions.

The application of Directive 2000/75/CE (9), through the adoption of Decision 2001/138/CE (4), disrupted animal trade in at least a third of Italy and, if sustained, would possibly have caused an irreversible decline in the cattle and smaller ruminants production sector.

Italy is a relatively small country (301 230 km^2), smaller than other industrialised countries (such as the United States, Australia or South Africa) that have experience of BT or incursions of BTV. The small size of Italy makes it difficult to apply the movement restrictions prescribed by the Directive without extensive changes to the structure of the Italian livestock industry, which is characterised by different levels of specialisation, with individual phases of the production cycle performed in different regions. It rapidly became evident after demarcation of the protection and surveillance zones, which involved about one third of the country, that this designation would have prevented the slaughter of cows and the fattening of calves concentrated in the free zone in the north of Italy. In addition, it would have been impossible to continue the practice of transhumance, because the traditional pathways along which livestock are moved cross the surveillance zones. Transhumance has been practised since pre-Roman times and is therefore embedded deeply in the livestock culture of Italy.

Potential vaccination strategies for BT include (30):

a) vaccination of cattle only
b) vaccination of both sheep and cattle
c) vaccination of sheep only.

Vaccination clearly reduces the number of susceptible animals; therefore, fewer animals will become viraemic following infection. Vaccination of cattle may be a more effective control measure than the vaccination of sheep, as viraemic cattle are more common than viraemic sheep and viraemic cattle are frequently the source of BTV. Vaccination of sheep can be effective in reducing the number of cases of BT; indeed, the large-scale use of attenuated vaccines in South Africa and Israel has made sheep farming possible in areas where it was previously uneconomical.

The three control strategies adopted in Europe and in the rest of the world can be summarised as follows:

1) direct, rather than preventive, control measures, such as those adopted in Greece
2) vaccination of sheep only (adopted in the Balearic islands and in Corsica); this reduces the impact of the disease but has no impact on virus circulation between cattle and vectors
3) vaccination of all susceptible ruminant livestock (cattle, buffalo, sheep and goats); this is the approach adopted by the Italian authorities and is designed to interrupt the natural cycle of BTV infection.

The vaccination strategy adopted in Italy was based on a risk assessment which suggested that direct economic losses would be reduced or eliminated and virus circulation would be reduced significantly (15). The risk assessment showed that when at least 80% of the susceptible populations has been immunised, the number of secondary cases should be less than 1% of the number expected to occur in the absence of vaccination. Thus, it was predicted that vaccination of all ruminants would not only reduce virus circulation and consequently reduce the size of the restricted areas, but also reduce the duration of movement restrictions. Existing literature on the use of BT vaccines is exclusively related to sheep; no studies address the vaccination of cattle against BT. Therefore, the application of the strategy suggested by the risk assessment required preliminary experimental field trials.

This paper describes research conducted in Italy prior to the commencement of the BT vaccination campaign and provides the results of the campaign itself.

Preliminary studies and field trials

A number of preliminary studies were conducted prior to the commencement of the vaccination campaign and during the initial BT vaccination campaign itself. The objective was to investigate potential adverse side-effects of BT vaccination in cattle and sheep, the kinetics of the antibody response in vaccinated cattle and sheep, and the effect of vaccination on clinical BT and viraemia in animals exposed to wild-type BTV in the field. Some of these studies were performed in controlled conditions and others in the field. The field studies were performed only in infected areas so as to limit the risk of virus dissemination connected with field trials.

A phylogenetic tree was compiled from the VP2 gene sequences of the Italian, Greek, Israeli and South African BTV serotypes 2, 4, 9 and 16 (BTV-2, BTV-4, BTV-9 and BTV-16), reference and field isolates of BTV, as well as of VP2 gene sequences currently available on GenBank. The Italian isolates were obtained from different regions, species (cattle, goats, sheep and deer) over different years (2000-2002). Phylogenetic analysis showed that all the Italian BTV-2 isolates grouped together and they were also identical to the strain of BTV-2 isolated in Corsica. There was 96% identity between the European BTV-2 isolates and the BTV-2 South African reference and vaccine strains. The analysis of Italian isolates of BTV-9 showed clearly that these isolates were almost all identical to the BTV-9 isolates from 2001, and they were highly homologous (99%) to BTV-9 isolate from Greece. In contrast, the VP2 genes of Australian and European isolates of BTV-9 had only 89% identity and the two groups of isolates shared only 67% identity to the reference BTV-9 isolate from South Africa (26).

The modified live-attenuated monovalent vaccine against BTV-2 elicited complete protection against challenge with $10^5 TCID_{50}$/ml of virulent homologous virus in cattle inoculated seven months after vaccination, without any detectable viraemia (23). The duration and titre of viraemia were also reduced in animals challenged 14 months after vaccination and viraemia in vaccinated animals was considered of insufficient titre to infect vector insects (G. Savini, personal communication). Despite the lower homology between vaccine and wild strains of BTV-9, vaccination protected sheep against challenge with $10^{5.8} TCID_{50}$/ml of virulent homologous virus of Italian origin, three months after vaccination. There was no detectable viraemia in vaccinated sheep after challenge (G. Savini, personal communication).

Median antibody titres in cattle two months after vaccination with monovalent vaccine BTV-2 were 1:160 (22). Median antibody titres in sheep, at 42 days post vaccination with BTV-2 monovalent vaccine were 1:42.5 (27) whereas titres at 42 days after vaccination with BTV-9 monovalent vaccine were as low as 1:5 (27). Cattle vaccinated with bivalent vaccine (BTV-2 and BTV-9) had median antibody titres of 1:160 against BTV-2, with 13% negative animals, and median antibody titres of 1:20 against BTV-9 with 23% negative animals (20).

Concerning the possible adverse effects of vaccination on reproduction, neither abortion nor teratogenic defects were observed in cattle immunised with the monovalent BTV-2 vaccine, either in controlled or in field conditions (18). Similarly, in a field trial in cattle, no adverse effect on reproduction was observed after vaccination with the bivalent BTV-2 and BTV-9 vaccine (16). The administration of monovalent BTV-2 vaccine (14) or bivalent BTV-2 and BTV-9 vaccine (19) to cattle in field conditions did not affect the quantity and quality (somatic cell count, protein and fat content) in milk. Similarly, the administration of monovalent BTV-2 vaccine to sheep in controlled conditions did not affect milk production (11); however, the administration of bivalent BTV-2 and BTV-9 vaccine to sheep caused a transient 30% decrease in production that persisted for about one week (24, 25).

Results of vaccination campaigns

Due to limited knowledge on the distribution of vectors and the epidemiology of BT in northern Mediterranean countries during the first epidemic of 18 August 2000-14 May 2001, coupled with a lack of the necessary vaccine doses, the Italian authorities adopted actions that mainly addressed monitoring of the disease and development of a surveillance system. A total of 6 869 outbreaks of BT were reported during this first epidemic. On 11 May 2001, following the collection of data and a risk assessment, the Italian Ministry of Health ordered the vaccination of all domestic ruminants susceptible to the infection (sheep, goats, cattle and buffalo) in infected and in areas at risk (17). Despite the Ministerial Order, virtually no ruminants were vaccinated during 2001. The result was another

6 807 outbreaks and 250 662 affected sheep, according to the risk assessment.

Vaccination of susceptible populations began in autumn 2002. However, in the majority of the regions and provinces involved, activity actually began in January 2002 (12) (Fig. 1). Vaccination in Italy was implemented using two different vaccines, according to the BTV types observed in the various zones: monovalent BTV-2 vaccine was used in Sardinia, Tuscany, and Latium and bivalent vaccine with serotypes BTV-2 and BTV-9 was used in the regions of southern Italy. Zones in which vaccination was practised were modified according to the spread of infection during 2002 (Fig. 2).

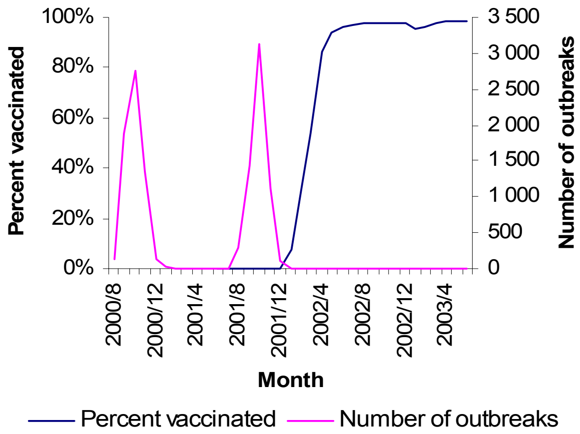

Figure 1
Percentage of population vaccinated against bluetongue and monthly number of outbreaks in Italy

When the new epidemic commenced in July 2002, the level of vaccination in susceptible populations varied greatly in the different regions. In July 2002, when the new epidemic peak commenced, 57% of the eligible animals had already been vaccinated (Fig. 1) but vaccination coverage in the various regions varied greatly (Figs 3-9). Approximately 90% of susceptible animals were vaccinated in Sardinia (Fig. 3) and Tuscany (Fig. 4) (97% in Sardinia and 87% in Tuscany) before the start of the new epidemic peak. In Basilicata, on the other side of Italy (Fig. 5) only 2% of the population was vaccinated before the start of the new epidemic peak, but 84% of the eligible population was vaccinated by the end of the year. In the other regions (Sicily, Latium, Calabria and Campania) (Figs 6, 7, 8 and 9), less than two-thirds of the population had been vaccinated by the end of 2002 (12).

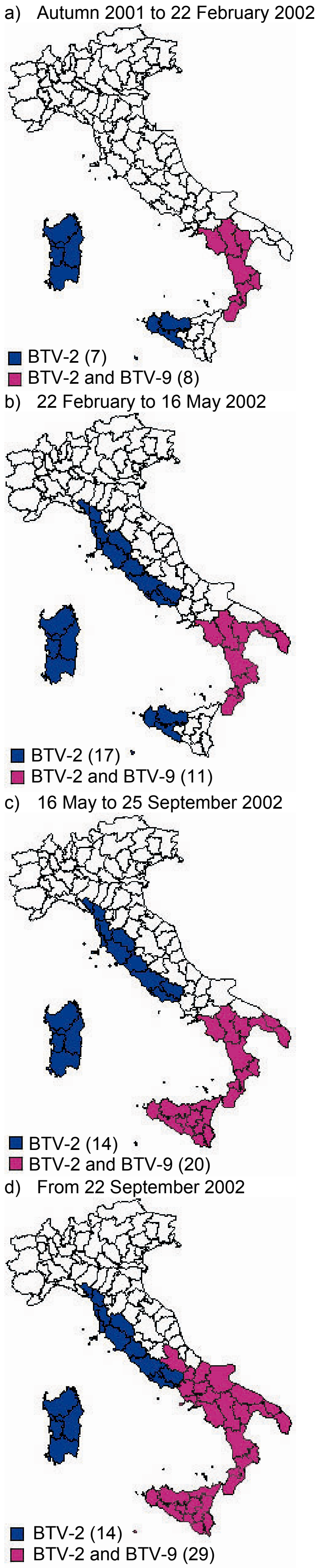

Figure 2
Evolution of bluetongue vaccination areas in Italy, 2002

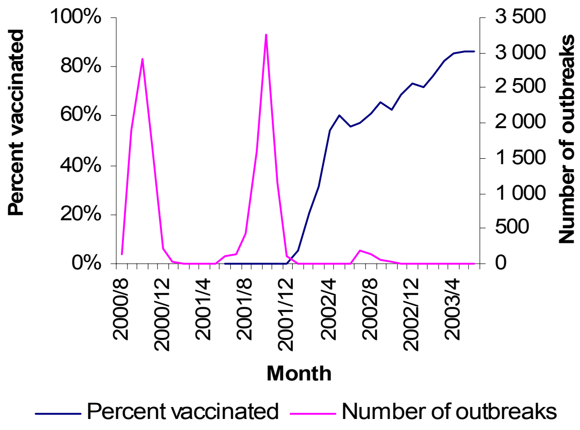

Figure 3
Percentage of population vaccinated against bluetongue and monthly number of outbreaks in Sardinia

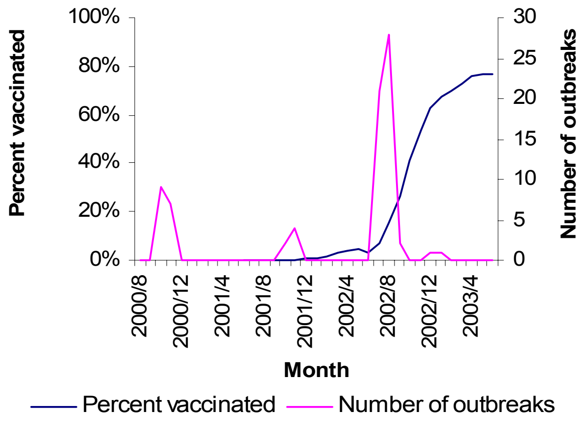

Figure 6
Percent vaccinated population and monthly number of outbreaks in Sicily

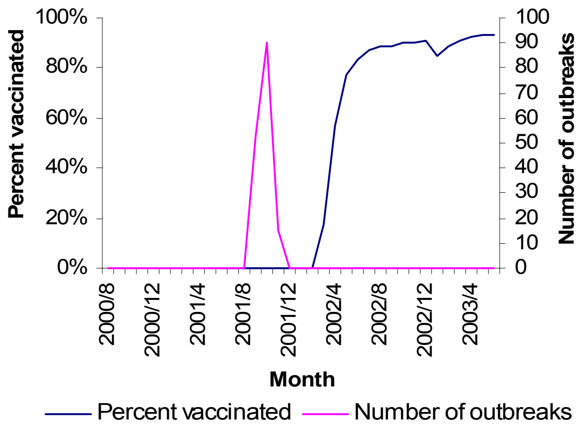

Figure 4
Percentage of population vaccinated against bluetongue and monthly number of outbreaks in Tuscany

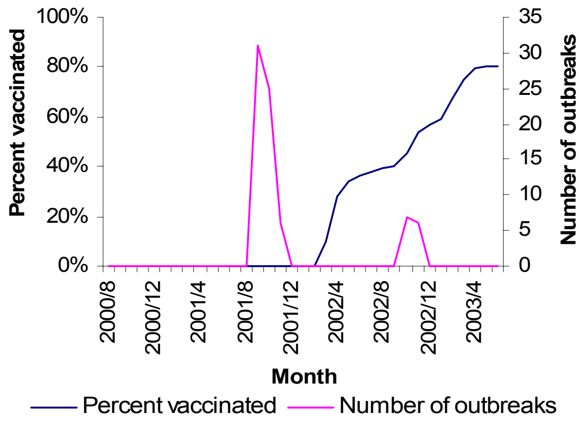

Figure 7
Percent vaccinated population and monthly number of outbreaks in Latium

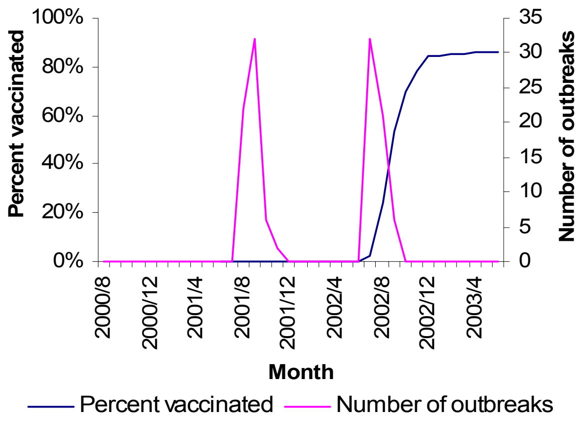

Figure 5
Percentage of population vaccinated against bluetongue and monthly number of outbreaks in Basilicata

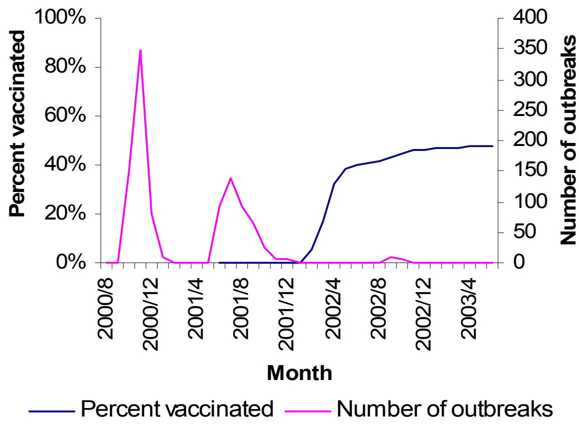

Figure 8
Percentage of population vaccinated against bluetongue and monthly number of outbreaks in Calabria

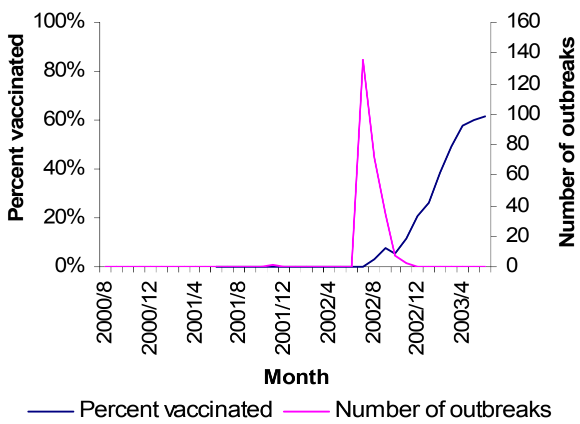

Figure 9
Percentage of population vaccinated against bluetongue and monthly number of outbreaks in Campania

The third BT epidemic commenced on 15 April 2002 and ended on 14 April 2003. During the 2002-2003 epidemic, infection due to both serotypes BTV-2 and BTV-9 spread to the province of Avellino (Campania) in July and to the provinces of Benevento and Caserta (Campania), Foggia and Bari (Apulia), L'Aquila (Abruzzo) and Isernia (Molise) in September. The only spread of BTV-2 to previously unaffected areas occurred in Massa (Tuscany) in September. The total number of outbreaks detected in the third epidemic was 427 in eight regions (Table I). The geographic distribution of the infection is presented in Fig. 10.

The different levels of vaccination had clear consequences on the occurrence of disease. In the two regions where approximately 90% of susceptible ruminants were vaccinated, clinical disease either disappeared (Tuscany, 158 outbreaks and 693 diseased animals in the 2001-2002 epidemic, 0 outbreaks in 2002-2003) (Fig. 4) or was reduced by a factor of 1/100 (Sardinia, 6 090 outbreaks and 239 178 diseased animals in the 2001-2002 epidemic, 10 outbreaks and 28 diseased animals in 2002) (Fig. 3). In the same regions, the spread of the infection was also substantially reduced by the vaccination campaign (Fig. 11). A clear demonstration of the efficacy of vaccination was shown in Sardinia where, in August 2003, a new epidemic due to BTV-4 occurred, causing

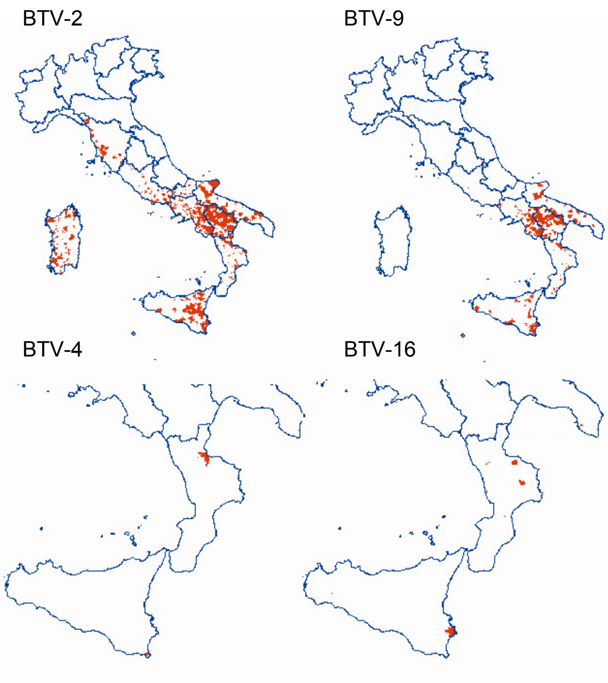

Figure 10
Geographical distribution of the infection in Italy, 15 April 2002-14 April 2003

Table I
Clinical outbreaks of bluetongue in Italy during the third epidemic,15 April 2002-14 April 2003

Region	Number of outbreaks	Total number of animals in infected flocks	Number of diseased animals	Number of dead animals	Number of slaughtered animals
Basilicata	59	5 145	223	96	–
Calabria	15	797	87	–	87
Campania	251	20 918	1 951	1 495	213
Lazio	14	1 702	44	37	–
Molise	13	2 781	5	8	–
Puglia	17	2 484	284	245	1
Sardegna	10	2 120	28	5	4
Sicilia	53	12 304	1 076	1 092	–
Total	427	47 404	3 531	2 953	305

850 outbreaks of BT in six weeks. This showed that the reduction in the circulation of BTV-2 was due to the immunity induced by the vaccine and not to the disappearance of the conditions conducive to spread of the virus.

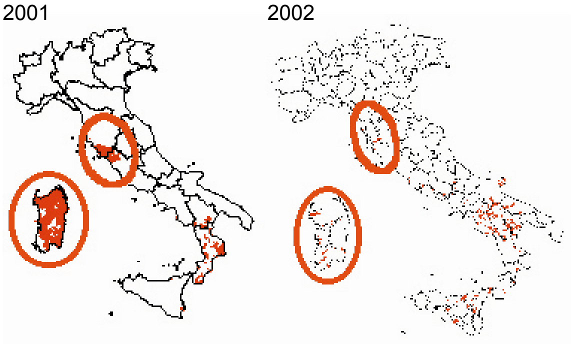

Figure 11
Infection spread in Sardinia and Tuscany before and after the vaccination campaign

Vaccination apparently did not significantly reduce the spread of either disease or infection in the five regions of central-southern Italy (Basilicata, Calabria, Campania, Latium and Sicily) where variable proportions of the eligible population were vaccinated before the beginning of the new epidemic. A total of 559 outbreaks were recorded in central and southern Italy prior to vaccination. In the 2001-2002 epidemic (after the introduction of vaccination), infection spread to two additional regions (Molise and Puglia) causing a total of 417 outbreaks. However, vaccination did limit direct losses in these regions, despite the spread of infection to neighbouring regions and a total number of outbreaks that was similar to the previous year. The number of outbreaks recorded in the 2002-2003 epidemic in the five regions of central and southern Italy was, in fact, significantly correlated to the level of vaccination achieved by each region at the end of July 2002 (Spearman's ρ= -0.9150, $p<0.0001$) (28).

It is concluded, therefore, that the vaccination of ruminants led to a progressive reduction of virus circulation and consequently of the zones in which movement restrictions were applied. In Sardinia, for example, a decrease in monthly seroconversion rates began in May 2002, when more than 90% of the susceptible population had been vaccinated (Fig. 12). The seroconversion rate was 6.8% in April 2002 and decreased to 4.8% by May, stabilising at approximately 4% in the following months, through to December 2002. The period between July and October when the monthly seroconversion rate fluctuated at around 4% corresponded to the epidemic peak of previous years. A further decrease in seroconversion rates occurred after January 2003 when the temperature was unfavourable to *Culicoides* activity and the second vaccination campaign commenced, with the monthly rate declining to 1% without any increase in April, the month in which the maximum seroconversion rate was recorded the previous year.

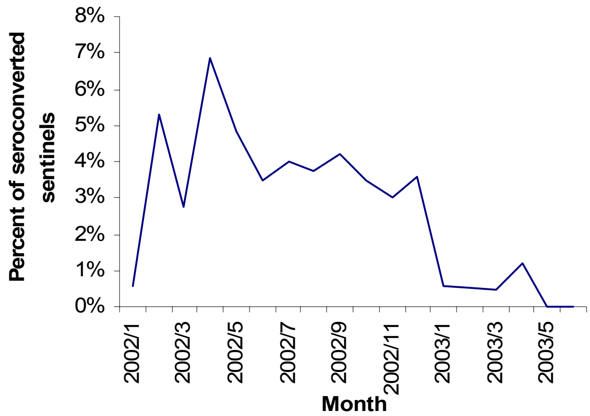

Figure 12
Percentage of sentinel population that seroconverted in Sardinia, January 2002-June 2003

The BT experience of Sardinia was used to evaluate the effect of vaccination on animal trade for the following reasons:

a) trade of cattle from Sardinia to continental Italy, especially to areas of northern Italy, was intensive prior to the BT epidemic
b) after the occurrence of BT in Sardinia, the export of cattle from the island to free areas of continental Italy came to a complete standstill
c) the progressive relaxation of movement restrictions opened access to the free areas of northern Italy and trade resumed.

The total number of cattle sent from Sardinia to continental Italy (Fig. 13) in 2002 was 1 019; 92% percent of these animals were moved during the last two months of the year when the effects of vaccination had been confirmed and wide areas of northern Italy were free from vectors. These areas on the mainland could therefore receive animals from the surveillance zones without the risk of losing their free status (5, 21). Towards the end of 2002 a new risk assessment (7, 13) led to:

a) the authorisation for movement of vaccinated animals directly for slaughter when at least 80% of the susceptible population was vaccinated
b) a new approach to define the areas under movement restrictions. From January to June 2003, a total of 3 097 cattle were sent from Sardinia to continental Italy, compared to 8 animals in the same period of the previous year.

The arrival and spread of BTV-4 to most of the island in August 2003 again halted exports from Sardinia.

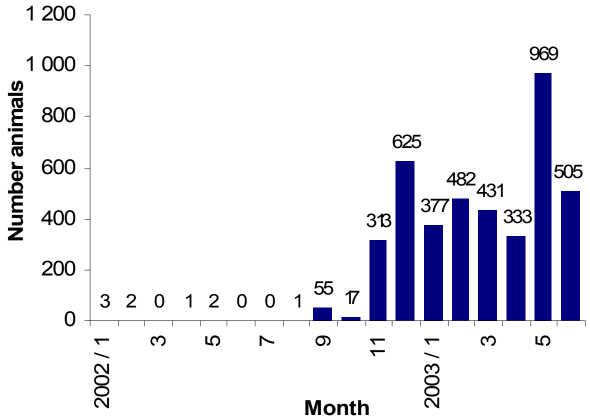

Figure 13
Animals exported from Sardinia to continental Italy, January 2002-June 2003

Monitoring of the possible side-effects of the BTV vaccine (death, abortion, stillbirth), was based on the following:

a) sampling of animals to determine the presence of the vaccine virus
b) collection of information concerning the vaccination itself (type of vaccine damage and dates of vaccination on the farm).

Samples were submitted for laboratory tests for the detection of vaccine and field strains of BTV. During the first vaccination campaign, there were few notifications of undesired vaccine side-effects (i.e. number of holdings requiring veterinary intervention and the collection of samples); only 312 of 87 245 holdings on which vaccination was applied were affected, representing 0.16% of cattle herds and 0.50% of small ruminant flocks vaccinated in Italy. Even fewer were confirmed by laboratory diagnosis (47 holdings, which is equivalent to 0.01% of vaccinated cattle herds and 0.09% of vaccinated sheep and goats flocks) (3). These field results were later confirmed by experimental studies on abortion and the teratogenic effects of the vaccine (16, 18).

A comparison can be made with data collected between 1991 and 2001 in the United States by the Vaccine Adverse Event Reporting System (VAERS). During the study period, 1.9 billion doses of 27 different types of human vaccines were administered and the prevalence of adverse events was 0.01% (29).

Discussion

Data collected during the successful vaccination campaign against BT in Italy led to the amendment of European Union legislation and ultimately to the adoption of the following measures:

1 From 10 January 2003 (Decision 2003/14/CE) (6), the despatch of slaughter animals from infected to free areas was allowed, provided that vaccination coverage included at least 80% of susceptible animals in the province of origin and that a risk assessment had been made

2) Decision 2003/218/CE (7) of 27 March 2003 introduced the concept of 'risk' into the European provisions and subdivided regions into areas of higher and lower epidemiological risk for BT. The decision, therefore, allows the despatch of live animals from the 'lower risk areas' where viral circulation has not been detected to all of the European Union, and the despatch of slaughter animals from 'lower risk areas' even those with active infection and from 'higher risk areas' where viral circulation has not been detected to free areas in the national territory. The latter is allowed only if the animals have been vaccinated at least 30 days prior to movement, they belong to a herd in which all the animals have been vaccinated, and transport occurs during daylight hours only. According to the Decision 2003/218/CE, the member state is free to demarcate 'epidemiological relevant areas of origin'. In other words, on the basis of surveillance results, it can reduce or increase the protection zone to a radius of greater or less than 20 km and can evaluate the possibility of demarcating lower risk areas in higher risk territories.

3) All existing European Union legislation regarding the compensation of farmers was developed in relation to contagious diseases of OIE 'List A', mainly foot and mouth disease and hog cholera (classical swine fever). In the case of an outbreak of such diseases, the control strategy in Europe relies on the stamping-out of infected and in-contact animals and, since 1990, vaccination is only an ancillary control measure. The principal economic losses in such a scenario are direct, and result from the slaughter of infected and in-contact animals; any compensation for indirect losses would represent, according to the European legislation, a disturbance of the market. In the case of vector-borne disease, especially when vaccination is the principal control measure, direct losses are virtually negligible but indirect losses due to movement restrictions become substantial. Moreover, losses incurred as a result of movement restrictions also have an

impact on farmers in free areas who are dependent on animal movement for their livelihoods as well. This has only very recently been recognised (July 2003) by the European Commission with the enactment of Decision C(2003)2519fin (8), authorising Sardinia to compensate farmers who suffered indirect losses due to movement restrictions between 6 September 2000 and 31 December 2001.

Based on the results of trials that evaluated the levels of viraemia after natural BTV infection of vaccinated animals, as well as results of the vaccination campaign (conducted between January and May 2003), a further risk assessment will be conducted to evaluate the possibility of trading vaccinated animals from areas in which virus circulation is still active. This risk assessment will also be relevant to international animal trade and might serve as the basis for a revision of existing international standards on BT.

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