Long-term persistence of vaccine-elicited bluetongue serotype 8 neutralizing antibodies in dairy cattle

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SUMMARY

Introduction - Bluetongue virus serotype 8 (BTV-8) caused a massive epidemic in Europe in 2006-2007. In response to this epidemic, large-scale vaccination campaigns of susceptible species were implemented in most European countries starting from the spring of 2008, when inactivated BTV-8 vaccines became available on the market. In Italy, the first evidence of BTV-8 circulation was reported in March 2008 from five dairy cattle herds in the province of Verona. Compulsory vaccination was then implemented in the two adjoining provinces of Verona and Mantua.

Aim - This study aims at determining the prevalence of vaccine-elicited BTV-8 neutralizing antibodies in dairy cattle 29-47 months after vaccination. As infected and vaccinated animals cannot be differentiated serologically, information on the post-vaccination antibody dynamic under field conditions is crucial to implement surveillance activities.

Materials and methods - A cross-sectional serosurvey of vaccine-elicited BTV-8 neutralizing antibodies in dairy cattle was performed in the province of Verona; 110 cows housed in 12 farms vaccinated during the 2008 campaign, and 270 cows housed in 28 farms vaccinated during both 2008 and 2009 campaigns, were sampled in 2012. Eighty-four non-vaccinated heifers born from 2010 onwards were also sampled to confirm absence of virus circulation in the sampled herds after vaccination. All samples were tested for BTV-8 neutralizing antibodies using virus neutralization assay. Differences in seroprevalence between cows receiving one or two vaccinations (2008 vs. 2008 and 2009) were tested with χ² test. The analysis considered stratification of samples according to herd size and vaccination schedule, clustering of animals at the herd level, and sampling weights as to weight the sample back to the population from which it was drawn.

Results and discussion - Overall seroprevalence was 43.1% (95% confidence interval: 41.8-44.5%), relatively low compared to that of similar surveys performed in other countries. Seroprevalence in cows vaccinated once was 30.4% (29.3-31.5%), significantly lower than that in cows vaccinated twice (48.3%, 46.5-50.2%). Antibody titres ranged from log₁₀ 1.00 to 2.51 (mean 1.36). None of the 84 non-vaccinated heifers had detectable BTV-8 neutralizing antibodies, confirming absence of virus circulation in the sampled herds after vaccination.

Conclusions - A seroprevalence of 43.1% was evidenced in Italian dairy cattle 29-47 months post-vaccination. Although neutralizing antibodies are being waned sooner in formerly, non-repeatedly vaccinated animals, for at least 47 months vaccinated animals cannot be included in (sero)surveillance programmes. The fact that many vaccinated animals are likely to still be protected by vaccination during that period provides an explanation of why the devastating 2006-2007 European BTV-8 epidemic was so efficiently controlled despite most vaccination campaigns were stopped after two years.

KEY WORDS
Bluetongue; BTV-8; humoral response; inactivated vaccine; Italy.

INTRODUCTION

Bluetongue is a midge-borne disease of ruminants caused by a virus of the family Reoviridae, genus Orbivirus. Twenty-six bluetongue virus (BTV) serotypes have been identified worldwide1. Amongst these, the BTV serotype 8 (BTV-8) caused a massive epidemic in Europe in 2006-20072. Although BTV-8 infection is far less manifested in bovines than ovicapines, it causes considerable economic losses in bovines as a consequence of the bans on international trade of infected animals. Under certain conditions (European Commission Regulation No. 1266/2007), vaccination allows for safe trade of live animals. To this purpose, large-scale vaccination campaigns for BTV-8 were implemented in most European countries starting from the spring of 2008, when inactivated BTV-8 vaccines became available on the market3.

In Italy, the first evidence of BTV-8 circulation was reported in March 2008 from five dairy cattle herds in the province of Verona3. Compulsory vaccination was implemented in the two adjoining provinces of Verona and Mantua. By autumn 2008, vaccine coverage amounted to 77% (Verona) and 87% (Mantua)3. A high level of vaccine coverage was also achieved in other countries3, apparently resulting in a marked reduction in BTV-8 incidence across Europe3.
As infected and vaccinated animals cannot be differentiated serologically, information on the post-vaccination antibody dynamic under field conditions is crucial to implement surveillance activities. Here we present the results of a cross-sectional serosurvey of BTV-8 neutralizing antibodies in Italian dairy cattle 29-47 months after vaccination.

MATERIALS AND METHODS

The study area comprised the whole province of Verona (3121 km²). Cattle aged ≥26 months were vaccinated with two shots of Zulvac® 8 Bovis inactivated vaccine, three weeks apart, according to manufacturer’s instructions. Of the 1044 vaccinated dairy cattle herds, 398 were vaccinated in June-December 2008 only, whereas 646 were vaccinated in June-December 2008 and then re-vaccinated in April-November 2009. In February 2012, when this study was set up, 25292 vaccinated dairy cows (out of 210638 bovines present in the study area) were still housed in 909 (out of 7149) cattle farms. The study consisted of a cross-sectional serosurvey. The sample size calculation assumed: 1) an expected prevalence of 50% in the vaccinated population (as the ‘worst-case scenario’); 2) a confidence level of 95%; and 3) 5% precision. Three-hundred and eighty target serum samples were taken from cows housed in 40 randomly selected dairy cattle farms (located all over the study area), which were proportionally allocated according to herd size (11-20, 21-30, 31-40, 41-50, 51-90, 91-150, and >150 animals) and vaccination schedule (2008 and 2008/2009) (Table 1). Sampling was performed in April-May 2012. Eighty-four non-vaccinated heifers born from 2010 onwards were also sampled to confirm absence of virus circulation in the sampled herds after vaccination. All samples were tested for BTV-8 neutralizing antibodies using virus neutralization assay as described elsewhere. Differences in seroprevalence between cows receiving one or two vaccinations (2008 vs. 2008 and 2009) were tested with χ² test (α-level: 0.05). A ‘design-based’ analysis was applied. This considered stratification of samples according to herd size and vaccination schedule, clustering of animals at the herd level, and sampling weights to as to weigh the sample back to the population from which it was drawn. Statistical analysis was performed using Stata 11.2.

RESULTS

Seroprevalences per herd size group and vaccination schedule are reported in Table 1. Overall seroprevalence was 43.1% (95% confidence interval [95%CI]: 41.8-44.5%). Seroprevalence in cows vaccinated twice was 48.3% (95%CI: 46.5-50.2%), significantly higher (p<0.001) than that measured in cows vaccinated once (30.4%, 95%CI: 29.3-31.5%). Antibody titres ranged from log10 1.00 to 2.51 (mean 1.36). None of the 84 non-vaccinated heifers had detectable BTV-8 neutralizing antibodies, confirming absence of virus circulation in the sampled herds after vaccination.

DISCUSSION AND CONCLUSIONS

The rapid control of the devastating 2006-2007 European BTV-8 epidemic by means of vaccination resulted in most immunization campaigns being stopped after two years. Notwithstanding the fear that the reduced vaccine coverage would have led to a recrudescence of the infection, BTV-8 incidence continued to decrease, with zero cases reported from North-Western Europe in 2010. Challenge studies have shown that BTV-8 inactivated vaccines are protective for at least one year, yet serum antibodies have been shown to persist in up to 97% of vaccinated cattle for at least four years post-vaccination, suggesting that cattle are likely to be protected for this time period. Our post-vaccination seroprevalence estimates were relatively low compared to those reported from the UK. However, these authors used a different vaccine (Bovilis® BTV-8) and a different cattle population, and they sampled only one farm. We also found positivities as high as 100% in five farms (data not shown), but these high point estimates were then counterbalanced by the others within the framework of the serosurvey, leading to seroprevalences that are representative of the entire study area and not only of a single herd. Seroprevalence in cows vaccinated in 2008 only (i.e. 40-47 months before sampling) was significantly lower than that found in cows vaccinated in both 2008 and 2009 (i.e. sampled 29-37 months after the last vaccination), suggesting that vaccine-elicited neutralizing antibodies are being waned sooner in formerly, non-repeatedly vaccinated animals.

Table 1 - Number of tested animals and respective percent positivities for BTV-8 neutralizing antibodies by herd size and vaccination schedule.

<table>
<thead>
<tr>
<th>Herd size</th>
<th>Vaccination in 2008 only</th>
<th>% positive animals (95% CI)*</th>
<th>Vaccination in 2008 and 2009</th>
<th>% positive animals (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥20</td>
<td>20 (4)</td>
<td>67.7% (61.4-74.0%)</td>
<td>35 (7)</td>
<td>49.2% (39.3-59.2%)</td>
</tr>
<tr>
<td>21-30</td>
<td>10 (2)</td>
<td>21.6% (17.5-25.7%)</td>
<td>30 (6)</td>
<td>63.8% (59.5-68.1%)</td>
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<tr>
<td>31-40</td>
<td>20 (2)</td>
<td>16.3% (11.9-20.6%)</td>
<td>50 (5)</td>
<td>54.2% (48.9-59.6%)</td>
</tr>
<tr>
<td>41-50</td>
<td>10 (1)</td>
<td>27.2% (6.7-65.2%)</td>
<td>30 (3)</td>
<td>13.4% (12.2-14.6%)</td>
</tr>
<tr>
<td>51-90</td>
<td>30 (2)</td>
<td>48.0% (47.8-48.3%)</td>
<td>75 (5)</td>
<td>39.4% (33.6-45.2%)</td>
</tr>
<tr>
<td>91-150</td>
<td>20 (1)</td>
<td>5.0% (0.1-24.9%)</td>
<td>20 (1)</td>
<td>45.0% (5.7-43.7%)</td>
</tr>
<tr>
<td>≥151</td>
<td>–</td>
<td>–</td>
<td>30 (1)</td>
<td>65.0% (47.2-82.7%)</td>
</tr>
<tr>
<td>Overall</td>
<td>110 (12)</td>
<td>30.4% (29.3-31.5%)</td>
<td>270 (28)</td>
<td>43.1% (41.8-44.5%)</td>
</tr>
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*95% confidence interval based on Taylor’s linearised standard error which considers clustering of observations at the farm level (primary sampling unit).

**95% binomial exact confidence interval. No clustering at the farm level is considered as 100% of observations come from the same farm.
In conclusion, a seroprevalence of 43.1% was evidenced in Italian dairy cattle 29-47 months post-vaccination. Although the likelihood for a vaccinated cow to be still seropositive was significantly lower in animals vaccinated once (i.e. during the 2008 campaign) than twice (i.e. during both 2008 and 2009 campaigns), it is clear that for at least 47 months vaccinated animals cannot be included in (sero)surveillance programmes. The fact that a consistent part of vaccinated animals is likely to be still protected during that period also provides an explanation of why the control of BTV-8 infection by means of vaccination was so effective despite most immunization campaigns were stopped after two years.

References