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Short communication

Evaluation of the humoral immune response in adult dairy cattle three years after vaccination with a bluetongue serotype 8 inactivated vaccine

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ABSTRACT

Despite the widespread use of bluetongue serotype 8 (BTV-8) inactivated vaccines across Europe from 2008 to 2011, two very practical questions remain unanswered about the length of persistence of group-specific antibodies in milk and serum post-vaccination and the duration of protection beyond one year post-vaccination. This study has firstly revealed that group-specific antibodies persist at high levels in milk and serum in the majority of cattle for at least 3 years post-vaccination, thus removing the option of using these animals in ELISA-based surveillance programmes. Secondly neutralising antibodies have been shown to persist in the majority of cattle for at least 3 years post-vaccination, indicating that the cattle are likely to be protected for this time period. This extended duration of protection may have contributed towards the rapid and efficient eradication of BTV-8 from many European countries, despite reducing levels of vaccine coverage.

1. Introduction

Since the unexpected introduction, rapid spread and subsequent successful control of bluetongue (BT) caused by bluetongue virus serotype 8 (BTV-8) in northern and western Europe from 2006 to 2011 many questions remain unanswered about how and from where the virus arrived and about which factors contributed towards the rapid and apparently successful control of the outbreak. Although it is clear that vaccination was likely to have played a very important role in the control of BTV-8, other factors such as the high numbers of naturally infected animals in some countries as well as climate and temperature may have also contributed.

The production of inactivated vaccines against BTV-8 by several companies in 2008 and the introduction of both compulsory and voluntary vaccination campaigns in affected countries across Europe resulted in a high level of vaccine coverage being achieved and the successful control and, in some countries, rapid eradication of the virus. The speed of control/eradication of BTV-8 did indeed take people by surprise and in some countries resulted in compulsory vaccination campaigns being stopped after one or two years. This resulted in some farmers choosing not to revaccinate their livestock in the second and third years of the outbreak. Many experts feared that the reduced vaccine coverage observed in the second and third year of the outbreak would result in a resurgence of the virus. However, despite the reduced levels of vaccine coverage, the amount of cases of BTV-8 reported across Europe continued to decrease dramatically with no cases of BTV-8 reported in northern and western Europe in 2010.

The commercially available BTV-8 inactivated vaccines have shown good safety and efficacy in both cattle and sheep, and studies have shown that they are protective for at least 1 year post-vaccination [1–6]. The length of protection produced by these vaccines, beyond 1 year, has so far not been evaluated.

Many countries across Europe are currently carrying out surveillance in order to gain freedom from BT. Currently there are no effective antibody detection ELISA assays available commercially that differentiate vaccinated from infected animals (DIVA), so the majority of surveillance is being carried out using the more expensive real-time RT-PCR technology. Knowing the length of time taken for antibodies to wane post-vaccination to a level where they are negative in the commercially available milk and serum-based ELISA tests would be extremely useful in order for countries to know when/if and at what time period post-vaccination they are able to use cheaper milk or serum-based ELISA tests, as opposed to real-time RT-PCR, in their surveillance programmes.

This study set out to assess, under field conditions, the status of the humoral immune response in adult cattle 3 years after an initial course of BTV-8 vaccination. The extent of antibody persistence in both milk and blood through ELISA testing, as well as the persistence of neutralising antibodies was measured 3 years after the primary course of vaccination. This will provide information on the length of persistence of antibodies post-vaccination in the blood and milk as well as the persistence of neutralising antibodies, which is likely to be linked to protection. It will also shed light

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Table 1
Persistence of antibodies, measured by ELISA and SNT, in the milk and blood of adult dairy cattle three years post-vaccination with a BTV-8 inactivated vaccine.

Cow number	Milk ELISA (% S/P)[a]	sELISA (% S/P)[b]	cELISA (% S/N)[c]	SNT $\log_{10}$
1	**Neg (60)**	Pos (118)	Pos (60)	Pos (1.17)
2	Pos (293)	Pos (185)	Pos (10)	Pos (2.51)
3	Pos (219)	Pos (193)	Pos (14)	Pos (1.90)
4	Pos (292)	Pos (192)	Pos (7)	Pos (2.08)
5	**Neg (8)**	**Neg (22)**	**Neg (122)**	**Neg**
6	Pos (246)	Pos (176)	Pos (14)	Pos (1.48)
7	Pos (279)	Pos (193)	Pos (10)	Pos (1.48)
8	Pos (100)	Pos (165)	Pos (20)	Pos (1.00)
9	Pos (237)	Pos (183)	Pos (12)	Pos (1.17)
10	**Neg (54)**	Pos (176)	Inc (76)	Pos (1.17)
11	Pos (256)	Pos (193)	Pos (10)	Pos (1.60)
12	Pos (306)	Pos (190)	Pos (7)	Pos (2.38)
13	Pos (306)	Pos (193)	Pos (7)	Pos (1.30)
14	Pos (290)	Pos (184)	Pos (11)	Pos (1.17)
15	Pos (306)	Pos (184)	Pos (7)	Pos (1.90)
16	Pos (259)	Pos (156)	Pos (11)	Pos (1.30)
17	Pos (153)	Pos (176)	Pos (20)	Pos (1.60)
18	Pos (279)	Pos (185)	Pos (11)	Pos (2.08)
19	Pos (306)	Pos (193)	Pos (8)	Pos (1.00)
20	Pos (208)	Pos (193)	Pos (8)	Pos (1.60)
21	Pos (139)	Pos (193)	Pos (12)	Pos (2.20)
22	**Neg (46)**	**Neg (10)**	**Neg (90)**	Pos (1.17)
23	Pos (301)	Pos (187)	Pos (6)	Pos (1.48)
24	Pos (287)	Pos (122)	Pos (8)	Pos (1.60)
25	Pos (276)	Pos (181)	Pos (8)	Pos (1.60)
26	Pos (303)	Pos (187)	Pos (10)	Pos (1.78)
27	**Neg (48)**	Pos (107)	Pos (50)	Pos (1.30)
28	Pos (305)	Pos (193)	Pos (7)	Pos (1.78)
29	Pos (249)	Pos (193)	Pos (7)	Pos (1.30)
31	Pos (293)	Pos (193)	Pos (8)	Pos (1.48)
31	Pos (293)	Pos (191)	Pos (7)	Pos (2.38)
32	Pos (258)	Pos (165)	Pos (8)	Pos (1.78)
33	Pos (306)	Pos (183)	Pos (7)	Pos (1.78)
34	Pos (306)	Pos (189)	Pos (7)	Pos (1.00)
35	**Neg (74)**	Pos (178)	Pos (14)	Pos (1.60)
36	Pos (291)	Pos (170)	Pos (10)	Pos (1.17)
37	Pos (208)	Pos (153)	Pos (23)	Pos (1.17)
28	Pos (199)	Pos (188)	Pos (12)	Pos (1.17)
39	Pos (243)	Pos (191)	Pos (8)	Pos (1.48)
40	Pos (210)	Pos (184)	Pos (9)	Pos (1.48)
Bulk milk (50 cattle)	Pos (294)			
Bulk milk (100 cattle)	Pos (297)			
Bulk milk (whole herd)	Pos (294)			

SNT, serum neutralisation test; Pos, positive; Neg, negative; Inc, inconclusive.
cELISA, competitive ELISA; sELISA, sandwich ELISA.
% S/N, percentage of negativity compared to the negative control.
% S/P, percentage of positivity compared to the positive control.
[a] Individual samples: negative ≤90%, inconclusive 90–110%, positive ≥110%, bulk milk samples: negative ≤30%, inconclusive 30–40%, positive ≥40%.
[b] Negative ≤25%, inconclusive 25–30%, positive ≥30%.
[c] Positive ≤70%, inconclusive 70–80%, negative ≥80%.

on whether or at what time point post-vaccination that it may be possible to use milk or serum-based ELISA testing in surveillance programmes across Europe.

2. Materials and methods

2.1. Cattle and experimental design

Forty (40) adult Friesian – Holstein cattle from a dairy farm in Surrey, UK were selected for the study. Throughout the BTV-8 outbreak in the UK in 2007 the counties that were most severely affected by BT were located towards the southeast of the country (East Anglia and Kent). There were no clinical cases of BT confirmed in Surrey throughout 2007 and out of over 20,000 cattle and sheep tested for BTV antibodies by ELISA prior to movement out of the protection zone in the winter of 2007/2008 only 89 (0.4%) tested positive, with the vast majority of the positive animals being from East Anglia and Kent (DEFRA epidemiological report, 2008 [13]). Despite extensive active and passive surveillance that was carried out in the UK from 2008 to 2010 no further evidence of BTV circulation was discovered, resulting in the UK being declared BTV free in the summer of 2011. Therefore it is considered highly unlikely that any of the cattle on the farm in Surrey would have been naturally infected with BTV-8 both before vaccination in 2007 or after vaccination between 2008 and 2011.

The cattle were vaccinated on two occasions 4 weeks apart (according to the manufacturer's instructions) in May and June 2008 with the Intervet-manufactured Bovilis-BTV-8 (Intervet, Germany) inactivated vaccine. The cattle were not revaccinated in 2009, 2010 or 2011.

The 40 cattle were sampled in June 2011. Whole blood (serum) samples and individual milk samples were collected. Bulk milk samples were also collected from the first 50 cattle milked, the first 100 cattle milked and the whole milking herd.

2.2. ELISA assays

The detection of BTV specific antibodies in serum was carried out using a competitive ELISA assay (cELISA, Pourquier