Colostral antibody protection and interference with immunity in lambs born from sheep vaccinated with an inactivated Bluetongue serotype 8 vaccine

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\textbf{A B S T R A C T}

Widespread vaccination programmes against Bluetongue virus serotype 8 (BTV-8), using inactivated vaccines, are being carried out across many countries in northern, western and southern Europe. This study investigates the extent and length of colostral antibody protection, as well as the degree of colostral antibody induced interference of the immune response to BTV-8, in sheep. Significantly lower titres of neutralising antibodies were transferred in colostrum to lambs born from sheep vaccinated once as opposed those vaccinated twice (single vaccine in the first year and a booster vaccine in the second year). On BTV-8 challenge, lambs born from sheep vaccinated on two occasions, with the second booster vaccine given approximately 1 month prior to lambing, were protected from clinical disease for up to 14 weeks. BTV-8 was isolated from 5 of the 22 challenged lambs, although only one of these lambs showed a transient rise in body temperature with no other clinical signs. Lambs born from ewes given a second booster vaccine 1 month prior to lambing, are likely to be protected from clinical disease for at least 14 weeks, whereas lambs born from ewes vaccinated once are likely to be protected for a shorter time. Colostral antibodies present in the 13–14-week-old lambs appeared to interfere with the humoral response to challenge virus. These results suggest that colostral antibodies may interfere with vaccination in lambs up to at least 14 weeks of age.

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1. Introduction

Bluetongue virus serotype 8 (BTV-8) entered northern Europe for the first time in 2006, survived over the winter period of 2006/2007 and spread rapidly in the summer and autumn of 2007 resulting in severe economic losses to the livestock industries of many countries in northern and western Europe including Germany, the Netherlands, France and Belgium\textsuperscript{[1,2]}. In direct response to this outbreak the European Commission recommended a mass vaccination strategy to control the spread of the virus and affected countries chose to use inactivated as opposed to live vaccines in their control programmes. Inactivated BTV-8 vaccines were produced by several vaccine manufacturers and these vaccines became commercially available in the spring of 2008. Due to the urgency of the situation and the importance of vaccinating susceptible livestock as early as possible in 2008 before circulation of the virus commenced, the vaccines were given provisional marketing authorisations with incomplete data available on efficacy and durations of immunity.

Many millions of animals have been vaccinated across Europe, but the extent and duration of colostral protection in lambs and calves born from vaccinated dams is not known. A key question is whether lambs born from BTV-8 vaccinated dams are protected for up to 14 weeks—the time that many lambs are kept prior to slaughter. Knowing the extent and length of colostral antibody protection in lambs born from vaccinated dams would enable farmers to decide whether or not to vaccinate lambs. This decision would depend on farming practises and how long lambs are routinely kept on the farm prior to slaughter. In the UK the majority of lambs are born in the Spring (March–April), it is thus very important that these lambs are protected during a high risk BTV transmission period when both Culicoides vector midges and BTV may be circulating (May–October).

There are different recommendations for the earliest age at which the commercially available inactivated BTV-8 vaccines should be administered, varying from 1 to 3 months. Until now, there are no published data supporting these recommendations, in particular which consider the degree with which colostral antibodies might interfere with responses to vaccination in lambs and...
calves born from vaccinated dams. There is a danger that vaccination of animals too young, while they still have circulating colostral antibodies, might result in a reduced response to the vaccine. This could lead to vaccinated livestock becoming susceptible to infection after colostral antibodies have waned, at a time of the year when midges are abundant and BTV is circulating. Data are required to ensure that farmers can vaccinate their ewes at the optimal time prior to lambing to achieve maximum levels of colostral antibody transfer, maximising the duration of colostral immunity, while also indicating when youngstock should then be immunised.

The objectives of this study were to measure the extent and length of colostral antibody protection in lambs born from single and twice vaccinated ewes and to assess the degree of any colostral antibody-associated interference with the neutralising antibody response to BTV-8 challenge. The overall aims were to help farmers to know if they need to vaccinate lambs going for early slaughter against BTV and, if they do, at what age they should do so.

2. Materials and methods

2.1. Animals and experimental design

2.1.1. Group 1: unvaccinated control group

Three unvaccinated poll Dorset sheep between 6 and 9 months of age, born from unvaccinated dams, were used as unvaccinated controls in the study.

2.1.2. Group 2: single vaccinated sheep and lambs

Thirty-two adult healthy pregnant Cheviot and poll Dorset sheep were selected for the study from a farm in East Anglia in the UK. All animals were tested negative for BTV antibodies by serology and for viral RNA by real-time RT-PCR prior to inclusion in the study. The ewes were vaccinated on 9/5/2008 with an inactivated BTV-8 vaccine. In total 45 lambs were born from these ewes between 30/5/2008 and 18/8/2009. The farmer checked carefully that all the lambs received colostrum. Samples were taken from the ewes and lambs on 4/7/08 when the ewes were 56 days post-vaccination (dpv) and the lambs were 16 and 36 days old and tested for BTV antibodies by a competitive ELISA (cELISA), a sandwich (double antigen) ELISA (sELISA) and a serum neutralisation test (SNT).

2.1.3. Group 3: double vaccinated sheep and lambs

Nineteen adult healthy pregnant Charollais and Charollais cross poll Dorset ewes were selected for the study from a commercial farm in Norfolk, UK. The ewes were vaccinated for the first time in May 2008 with an inactivated BTV-8 vaccine. The ewes were then revaccinated on 15/11/08. The second BTV-8 vaccine was given at the same time as the Heptavac-P clostraliod vaccine (Intervet). In total 22 lambs were born from these ewes between the 10/12/2008 and the 19/12/2008 (i.e. 25–34 days after the second vaccine). The farmer checked carefully that all the lambs received colostrum. Samples were taken from the ewes on 18/2/2009 (95 days after the second vaccination) and from the lambs at two time-points when they were between 6 and 10 weeks old and tested for BTV antibodies by cELISA, sELISA and SNT.

2.1.4. BTV-8 challenge study

Twenty-two lambs from group 3, aged 12–13 weeks old, along with 3 unvaccinated sheep from group 1 were bought into the BSL-3 animal facility at the Veterinary Laboratories Agency (VLA), Weybridge, UK, approximately 1 week before challenge. The lambs were inoculated subcutaneously in the neck with 1 ml of the Netherlands 2006 strain that had been passaged twice in Culicoides variipennis (KC) cells (NET2006/02). This BTV challenge protocol avoids the use of virus grown on mammalian cells as this has been found to reduce viral virulence. Due to a lack of cytopathic effect (cpe) in KC cells, viral titration of the inoculum dose was not possible but it gave a Ct of 15 when tested by real-time RT-PCR. EDTA and whole blood (serum) samples were taken from all the lambs on days −1, 2, 4, 6, 8, 10, 14, and 18 and 22 post-challenge infection (dpi). Samples were tested throughout the study by real-time RT-PCR, virus isolation, ELISA and serum samples were tested at −1 and 22 dpi by SNT. Throughout the study the body temperature of the animals was monitored daily and the sheep were examined by a veterinarian daily for clinical signs.

2.2. Vaccine

The sheep were vaccinated subcutaneously with the Intervet manufactured Bovilis-BTV-8 inactivated vaccine according to the manufacturer’s instructions.

2.3. Molecular tests

2.3.1. Real-time RT-PCR

RNA was extracted from EDTA blood using either the MagnaPure (Roche) extraction robot using the ‘total NA/External lysis’ protocol. Real-time RT-PCR was performed using a modified version of the procedure described by Shaw et al. [3].

2.4. Serology tests

2.4.1. ELISA assays

Whole blood samples were centrifuged at 2400 × g for 5–10 min to obtain serum. The detection of BTV specific antibodies in serum was carried out using a competitive ELISA assays (Pourquier C-ELISA kit (IDEXX, UK) and a sandwich (double antigen) ELISA (ID-Screen Bluetongue Early detection ELISA, ID-Vet, France) according to the manufacturer’s instructions.

2.4.2. Serum neutralisation test (SNT)

SNT was performed according to the method of Haig [4] using the BTV-8 South African reference virus and serotype-specific BTV-8 positive control antisera. Briefly, sera were diluted (1:10 to 1:1280) and titrated against 100 TCID_{50} of the BTV-8 South African reference virus. Plates were incubated for 1 h at 37 °C and then transferred to 4 °C overnight. The following day 50 μl of a Vero cell (African green monkey kidney) suspension 2 × 10^{5}/ml were added per well and, after incubation for 4–7 days at 37 °C, the wells were scored for cytopathic effect (CPE) observed. The neutralisation titre was determined as the dilution of serum giving a 50% neutralisation end point.

2.5. Virus isolation

EDTA blood samples were washed 3× with PBS and sonicated [5]. KC (C. variipennis) cells were inoculated with 200–500 μl of washed blood and incubated overnight at 26 °C. The following day the inoculum was removed and replaced with fresh media (Schneiders (Sigma, UK), 1% pen/strep, 1% Amphotericin B, 10% FCS). Cells were incubated 26 °C for 7 days and then harvested by centrifugation (2400 × g for 5–10 min), supernatant was tested by real-time RT-PCR for the presence of BTV RNA as described in Section 2.3.

2.6. Statistical analyses

Peak levels of viraemia as determined by real-time RT-PCR were compared statistically between unvaccinated group 1 lambs and the levels observed in the 5 lambs that became viraemic. Serological responses to infection, determined by observing an increase in antibody levels, were compared between colostrally fed lambs and
unvaccinated controls. The Wilcoxon Rank Sum test, using the R statistical package, was used for both comparisons and statistical significance was set at $p \leq 0.05$.

3. Results

3.1. Transfer of colostral antibodies to lambs born from ewes vaccinated once with a BTV-8 inactivated vaccine

Of the 32 once vaccinated ewes in group 2, only 43% developed antibodies detectable by SNT or cELISA, but 90% had antibodies detectable by sELISA (Table 1). The neutralising antibody titres were low ($\log_{10} 1\text{–}1.6$) in the SNT positive ewes and none of the 16–36-day-old lambs born from these single vaccinated ewes had cELISA antibodies detectable, 31% had sELISA antibodies and only 6% of the lambs had detectable neutralising antibodies (Table 1).

3.2. Transfer of colostral antibodies to lambs born from ewes vaccinated on two occasions (2nd dose 25–34 days prior to lambing)

In the 19 twice vaccinated ewes from group 3, all had antibodies at 95 days post-second vaccination using cELISA, sELISA and SNT (Table 2). All lambs born from these double vaccinated ewes had both SNT and sELISA antibodies at between 6 and 10 weeks old. At 13–14 weeks old, all lambs were positive by sELISA, but neutralising antibody levels had declined (Table 2). Titres of neutralising antibodies in the ewes at 95 dpv and in the lambs at both 6–10 weeks old and 13–14 weeks old are shown in Fig. 1.

3.3. Colostral antibody protection in lambs born from ewes vaccinated on two occasions (2nd dose 25–34 days prior to lambing)

Following challenge with BTV-8, viral RNA was detected in the blood of control unvaccinated animals from 2 dpi (Fig. 2a). The levels of BTV RNA, as measured by the threshold cycle ($C_t$), peaked

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Ewes (56 dpv)</th>
<th>Lambs (16–36 days old)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>32 ewes</td>
<td>45 lambs</td>
</tr>
<tr>
<td>cELISA</td>
<td>14/32 (43%)</td>
<td>0/45 (0%)</td>
</tr>
<tr>
<td>sELISA</td>
<td>29/32 (90%)</td>
<td>14/45 (31%)</td>
</tr>
<tr>
<td>SNT</td>
<td>14/32 (43%)</td>
<td>3/45 (6%)</td>
</tr>
</tbody>
</table>

sELISA: sandwich (double antigen) ELISA; cELISA: competitive ELISA; SNT: serum neutralisation test; dpv: days post-vaccination.

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Ewes (dams)</th>
<th>Lambs (6–10 weeks old)</th>
<th>Lambs (13–14 weeks old)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>19 ewes</td>
<td>22/22</td>
<td>9/22</td>
</tr>
<tr>
<td>cELISA</td>
<td>19/19</td>
<td>22/22</td>
<td></td>
</tr>
<tr>
<td>sELISA</td>
<td>19/19</td>
<td>22/22</td>
<td>14/22 (low titre)</td>
</tr>
<tr>
<td>SNT</td>
<td>All positive (high titre)</td>
<td>All positive (mod/low titre)</td>
<td></td>
</tr>
</tbody>
</table>

sELISA: sandwich (double antigen) ELISA; cELISA: competitive ELISA; SNT: serum neutralisation test.
at around 4–6 dpi and then slowly reduced up to 21 dpi when the study was terminated (Fig. 2a). Virus was isolated from blood from all 3 unvaccinated controls from 4 dpi up to 21 dpi. These control sheep showed mild to moderate signs of BTV, including inappetence, facial oedema, hyperaemia of the gums, ulcerous lesions in the mouth, nasal discharge and laboured breathing and all had elevated body temperatures (Fig. 3).

All the 22 colostrum-fed lambs in group 3 were protected clinically from BTV-8 challenge at 13–14 weeks old, although 1 of the 22 lambs exhibited a transient rise in body temperature above 40°C at 6 dpi. The average body temperatures of the 3 unvaccinated lambs (group 1) and the 22 lambs (group 3) are shown in Fig. 3. Five of the colostrum-fed lambs developed a viraemia and viral RNA was detected by real-time RT-PCR (Fig. 2b). Lower peak levels of viral RNA (as measured by Ct values) were seen as compared to the unvaccinated control group (Fig. 2, \( p = 0.002 \)).

Neutralising antibody titres in the unvaccinated control sheep (group 1) and the 22 colostrum-fed lambs (group 3) were measured by SNT on the day prior to challenge and at 22 dpi (Table 3). In the 3 unvaccinated control sheep, neutralising antibodies increased from undetectable levels to high levels at 22 days post-challenge. Fourteen of the 22 lambs in group 3 had circulating neutralising antibodies at the time of challenge and were fully protected both clinically and virologically from BTV-8 challenge. Antibody titres in 12/14 of these lambs declined over the period. Three of the lambs with undetectable levels of neutralising antibody titres on challenge were also protected both clinically and virologically and one of them seroconverted. One of the lambs with circulating neutralising antibodies had a significant increase in circulating antibodies, despite viral RNA not being detected in blood. The remaining 5 lambs (bold in Table 3) had no detectable neutralising antibodies at challenge, became BTV RNA positive by RT-PCR and virus isolation positive after challenge and also developed significant increases in neutralising antibody titres (Table 3). Statistical comparison of neutralising antibody levels after challenge in the colostral fed lambs that had become infected, as denoted by any increase in antibody levels (accompanied in some by detection of viral RNA) with the control group demonstrated that the serological response was significantly reduced in lambs which had received some colostral immunity (Table 3, \( p = 0.04 \)), despite the small sample size.

### 4. Discussion

After the unexpected incursion of BTV-8 into northern Europe in 2006 and the subsequent spread of the virus to neighbouring countries in 2007 and 2008, many vaccine manufacturers responded by rapidly producing inactivated BTV-8 vaccines. These vaccines were licenced for use under emergency regulations without any guarantees of efficacy and without knowing the length of the protective period, the extent and length of colostral antibody protection in lambs and the levels of interference of vaccination caused by colostral antibodies. Subsequent to release, three of the inactivated BTV-8 vaccines have demonstrated good efficacy and safety [6] and a separate inactivated BTV-8 vaccine was found to give good protection from clinical disease up to 10 months after a single vaccination in sheep [7].

Some vaccines companies recommended an initial single dose vaccination strategy in sheep whereas other companies recommended an initial course of two vaccines 1 month apart. Although inactivated vaccines have been used successfully in Europe for many years to control BTV infections there is very little known about the extent and length of colostral antibody protection in lambs born from vaccinated dams and the extent of colostral antibody related interference of the immune response to BTV vaccines. Many lambs are sold for meat production when they are around 14 weeks old, making it very important for farmers to know if their lambs are protected by colostral antibodies for this time period. If a vaccine strategy could be implemented so that lambs were likely to be protected for the time period required to fatten the lambs, this would mean that vaccination of the lambs would not be necessary, saving the sheep industry a considerable amount of money. If however the duration of colostral antibody protection was shorter than 12–14 weeks it would be necessary to vaccinate the lambs as they would be alive and susceptible to BTV during a high risk period when both Culicoides vector midges and BTV will be circulating (May–October).

This study was designed to fit in with common sheep farming practices whereby sheep are routinely vaccinated approximately 1 month before lambing with a combined clostridial/pasteurella...
vaccine in order to optimise levels of colostral antibody protection against these pathogens in lambs. Many sheep farmers in the UK expressed the wish to vaccinate their ewes at the same time with a BTV-8 inactivated vaccine in order to avoid the sheep having to be vaccinated on two occasions while pregnant, thus reducing the level of handling which could lead to stress-related abortions.

This study revealed a marked difference in the levels of neutralising antibodies in lambs suckled by ewes vaccinated on a single occasion with a BTV-8 inactivated vaccine as compared to lambs born from sheep vaccinated on two occasions (single vaccine in the first year and a booster vaccine in the second year), with the second booster vaccine given approximately 1 month prior to lambing. Low or no detectable neutralising antibodies were present in the majority of lambs born from sheep vaccinated once, indicating that these lambs may be unprotected or only protected for a limited time period after birth. In contrast high levels of neutralising antibodies were seen in lambs born from sheep vaccinated twice indicating that these lambs are likely to be protected for longer periods of time.

In order to investigate the extent and length of protection in the lambs born from ewes that had been given a single vaccination in the first year followed by a booster vaccination the next year approximately 1 month prior to lambing, 22 lambs were challenged with BTV-8 at 13–14 weeks of age. This age-group of lambs was chosen for the study as this is the age when many lambs are reaching slaughter weight and would be killed for meat production. No clinical signs were seen in any of the 22 lambs after direct challenge at 13–14 weeks of age, but a transient pyrexia in one. Seventeen of the 22 lambs (77%) were also protected from viral replication, but 5 of the lambs exhibited some degree of virus replication post-challenge, suggesting that these lambs might be able to transmit virus to a local Culicoides midge population.

All of the 14 lambs with neutralising antibody titres on the day prior to challenge were fully protected both clinically and virologically and another 3 animals with no detectable circulating neutralising antibodies were also fully protected. However, none of the 5 lambs that developed a viraemia had detectable neutralising antibodies at challenge. This is consistent with previous studies in that vaccinated animals with neutralising antibodies are generally protected against infection, but not all vaccinated animals with no neutralising antibodies are fully susceptible [7–10].

At the present time there are markedly different recommendations as to the earliest age at which lambs should be vaccinated with the commercially available BTV-8 inactivated vaccines. Some companies recommend vaccination of lambs from 4 weeks of age and some from 3 months of age. There are currently no published data describing the degree of colostral antibody induced interference of vaccination in lambs born from vaccinated dams. This information is vital to farmers as, if levels of colostral antibody interference were high, a vaccine given to lambs early in life may not be effective. It is important to identify the optimal age to vaccinate stock which will be at a time when colostral antibodies have reduced to a level where they do not interfere with vaccination. Data presented in this paper show that titres of neutralising colostral antibodies are lower and persist for a shorter duration in lambs born from single rather than twice vaccinated ewes. Therefore it is likely that the levels of interference will be lower and of shorter duration in lambs born from single compared to twice or more vaccinated sheep.

In 12 lambs that had neutralising colostral antibodies on the day prior to challenge, the titres of neutralising antibodies reduced between 0 and 22 days post-challenge, consistent with an expected reduction of colostral antibodies with time. It appeared that, in these 12 lambs, there was neutralisation of the challenge virus. The 5 lambs in which viral replication was detected showed increases in neutralising antibody titres lower than those in the unvaccinated control group. The apparent colostral antibody induced interference seen in these 13–14-week-old lambs merits further investigation by vaccine companies in order to see if similar levels of interference are seen in lambs/calves vaccinated with killed BTV vaccines. This work is essential in order to identify the optimum age to recommend vaccination of calves and lambs. This time period is likely to be different in lambs born from single as opposed to multiple vaccinated sheep due to the different amounts of colostral antibodies transferred to the lambs.

In conclusion, although further more detailed studies are required with more animals, this investigation has shown that the extent and length of colostral antibody protection in lambs is likely to be lower and shorter after the first year of vaccination, compared to when the dams have received a second booster vaccine dose. Significantly lower levels of colostral antibodies were transferred to the lambs from dams vaccinated once compared to twice. Lambs born from sheep vaccinated twice, with the second vaccination given approximately 1 month prior to lambing, are likely to be protected from clinical signs of BTV until they are at least 14 weeks old. Interference of the neutralising antibody response to challenge with BTV-8 in these lambs, born from twice vaccinated dams, was high. In the light of this investigation further detailed studies are necessary in order to investigate whether the current recommendations for the earliest age of vaccination are correct. Similar questions should also be addressed in cattle.

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