Preventive Veterinary Medicine 136 (2017) 49–55

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed

Efficacy of vaccination for bluetongue virus serotype 8 performed shortly before challenge and implications for animal trade

Massimo Spedicato, Alessio Lorusso*, Romolo Salini, Annapia Di Gennaro, Alessandra Leone, Liana Teodori, Claudia Casaccia, Ottavio Portanti, Paolo Calistri, Armando Giovannini, Giovanni Savini

OIE Reference Laboratory for Bluetongue, Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise (IZSAM), Campo Boario, 64100, Teramo, Italy

ARTICLE INFO

Article history:
Received 16 September 2016
Received in revised form 21 October 2016
Accepted 21 November 2016

Keywords:
Bluetongue virus serotype 8
Vaccine
Sheep
Animal trade
Emergency vaccination

ABSTRACT

Vaccination is the most effective strategy for controlling Bluetongue virus (BTV) spread and economic consequences thereof. In this study we verified in sheep, using one commercially available inactivated vaccine for BTV-8 (BTVPUR AlSap 8), when, during the recommended vaccination schedule, animals start to be effectively protected against challenge with wild-type strain. To this aim, sheep were challenged at different time points shortly after the first vaccine injection. Twenty-four Sarda sheep were divided into four groups vaccinated two weeks before challenge (Group A), one week before challenge (Group B) and concurrently with challenge (Group C). A second vaccine was performed twenty-eight days later with respect the first vaccine administration in each experimental group. The last group consisted of six non vaccinated-infected animals (NVIA). Virological and serological examinations were performed before and after challenge up to 42 and 77 days post challenge, respectively. The results of the study show that vaccination commenced as little as two weeks before challenge (Group A) prevented viremia and RNAemia in challenged sheep altogether. Conversely, Group B was partially protected from challenge and Group C showed viraemia and RNAemia similar to NVIA. This study indicates that the first administration of inactivated vaccine performed two weeks before challenge was able to prevent viraemia. Overall, our findings may have direct consequences for the management of an unexpected BTV-8 outbreak in sheep and for the legislation on sheep trade from BTV restriction areas.

1. Introduction

In 2006, an unexpected outbreak of Bluetongue virus (BTV) serotype 8 occurred in north Western Europe, in an area including Belgium, France, Germany, Luxembourg and the Netherlands (Dal Pozzo et al., 2009; Elbers et al., 2008a; Elbers et al., 2008b; Saegerman et al., 2008; Toussaint et al., 2006; Velthuis et al., 2010). The circulation of the same virus was also reported in northern Italy in 2008 and 2009, in regions with high levels of trade in livestock with the infected countries of northern Europe (http://bluetongue.izs.it/pls/izs_bt/bt_gestmenu.bt_index;Calistri et al., 2010; Caporale and Giovannini, 2010). Unlike other BTV serotypes that have been circulating in Europe, BTV-8 was causing disease in cattle and sheep. Bluetongue (BT) has severe economic repercussions for the livestock industry (Velthuis et al., 2010) due to direct losses caused by the infection but also due to indirect losses as a result of restrictions on animal trade (Dal Pozzo et al., 2009; Méroc et al., 2009; Nusinovici et al., 2013; Zientara and Ponsart, 2014; Tago et al., 2014).

A mass vaccination of cattle and sheep against BTV-8 started only in spring 2008 resulting in the progressive disappearance of viral circulation in 2009 and 2010 (Baetza, 2014; Garigliany et al., 2011; Zientara et al., 2010). However, in the late summer of 2015, BTV-8 has been reported again in some central provinces of France (Sailleau et al., 2015), threatening once again the livestock industry of the entire EU.

Vaccination is the cheapest effective measure to prevent the spread of the BT infection and consequences thereof (Patta et al., 2004; Savini et al., 2007; Zientara and Sánchez-Vizcaíno, 2013; Lorusso et al., 2013). Inactivated vaccines like those used for BT, in general, require two injections and the induced immunity is not long lasting (Savini et al., 2008).

As for animal movement, the EU legislation (Annex III of the Commission Regulation EC no. 1266/2007) considers the

* Corresponding author.
E-mail address: a.lorusso@izs.it (A. Lorusso).

http://dx.doi.org/10.1016/j.prevetmed.2016.11.016

exemption from the exit ban from restriction zones only when animals are vaccinated against the circulating serotype/s more than 60 days before the expected day of movement. Taking into account that inactivated BTV vaccines are administered twice 3–4 weeks apart, the period from vaccination to animal trade seems to be unnecessary long. However, some exceptions within some Member States of the EU do exist (Italian Ministry of Health no. 26559/2015 and 02010/2016; https://bluetongue.izs.it/j6_bluetongue/openDocument/?id=49 https://bluetongue.izs.it/j6_bluetongue/openDocument/?id=56). Italy, for example, permits to import susceptible livestock from restriction zones of Austria and France 10 days after the accomplishment of the vaccination schedule. This reduced waiting time was suggested by common sense; however, its effectiveness and equivalence to the procedure in the legislation in force have never been assessed.

Therefore, in this study we verified in sheep, using one commercially available inactivated vaccine for BTV-8 (BTVPUR AlSap 8), when, during the recommended vaccination schedule, animals start to be effectively protected against challenge with the wild-type strain. As far as we know, one licensed vaccine is declared by the manufacturer to be effective after a single injection in sheep (Bovilis BTV8), whereas previous studies demonstrated protection by other inactivated vaccines even after one single injection (Eschbaumer et al., 2009; Wäckerlin et al., 2010; Berry et al., 1982; Parker et al., 1975; Di Emidio et al., 2004). In particular, BTVPUR AlSap 8 has been shown to protect sheep after a single dose when challenged three months after vaccination (Eschbaumer et al., 2009). An additional study, also showed that antibodies produced by one single shot of this vaccine lasted for one year after immunization (Wäckerlin et al., 2010).

However, our study differs from those in published literature because we evaluated the earliest time-point in which sheep are fully protected. To this aim, sheep were challenged at different time points shortly after the first vaccine injection. Here we also discuss the potential implications of the obtained results for animal trade and the management of an unexpected BTV8 outbreak in sheep.

2. Materials and methods

All procedures on animals have been accomplished in compliance with the Italian Legislative Decree n.26/2014 on the protection of animals used for scientific purposes. The experiment was approved by the Italian Ministry of Health and by the Animal Welfare Committee of the Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise of Teramo (IZSAM).

2.1. Animals

Twenty-four clinically healthy Sarda sheep, between 15 and 23 months of age, were housed in insect-proof barns of the IZSAM. Upon arrival, all experimental animals were allowed to acclimatize for two weeks. Food and water were available ad libitum. Automatic temporized dispenser of pyrethroids operated throughout the entire experiment and black-light traps were in function both inside and outside the barns. Moreover, sheep were treated monthly with deltamethrin (Butox pour on, MSD Animal Health, Milan, Italy). The animals were numbered and randomly divided into four groups (divided into four different pens) including Groups A, B, C and Group K (non-vaccinated-infected animals, NVIA). Prior to vaccination, all animals tested negative for the presence of BTV RNA and BTV antibodies by real-time RT-PCR (Hofmann et al., 2008) and cELISA (Lelli et al., 2003), respectively.

2.2. Design of the experiment

The first vaccination of the immunization schedule was performed in Groups A and B, 14 (d-14) and 7 (d-7) days before challenge, respectively, and to Group C at the same day (d0) of the challenge. A booster vaccination was given to Groups A, B and C 28 days after the corresponding first vaccination (d14, d21 and d28 after challenge, respectively). The booster vaccination after the challenge in the three groups was performed to follow the manufacturer's instructions and was also aimed at assessing whether the booster could have a significant increase in the antibody titre.

NVIA were sham-vaccinated with 1 ml of sterile medium in place of the vaccine at the same time of Group C (d0). Rectal temperature, injection site, and clinical signs were monitored daily for the entire duration of the experiment. A rectal temperature $\geq$40 °C was considered as fever. Passage 2 on Vero cells of strain BTV-8/NET 2006 (BTV–8 wt), kindly provided by Kris de Clercq (CODA-CERVA-Belgium), was used for challenge infection. Before challenge, the virus was titrated by standard end-point (Reed and Muench, 1938) on African green monkey kidney cells (Vero), and subsequently suspended in MEM to obtain an infection dose of 10^6 $TCID_{50}$ ml^{-1}. Each animal of Groups A, B and C were administered with 1 ml (10^6 $TCID_{50}$ ml^{-1}) of the BTV–8 wt virus by the subcutaneous route in the neck. Whole blood was collected three times a week for six weeks (42 days) whereas serum samples were collected once a week for 11 weeks (77 days), starting from the day of the challenge.

Vaccination was performed with 1 ml of a licensed inactivated BTV-8 vaccine (BTVPUR AlSap 8, MERIAL, Lyon, France) according to the manufacturer's instructions. The vaccine contained 7.6-log10 $TCID_{50}$ of inactivated antigen (equivalent to titre prior to inactivation) and it is adjuvanted with saponin and aluminium hydroxide. The vaccines were injected subcutaneously on the left (first vaccination) and right (booster vaccination) side of the neck.

2.3. Laboratory tests

Antibody response was monitored by cELISA (Lelli et al., 2003). Neutralizing antibody were determined by serum-neutralization (SN) as previously described (Savini et al., 2004). BTV-8 RNA was detected using a one-step real time RT-PCR targeting segment 10 of the viral genome (rt-PCR_{BTV}) (Hofmann et al., 2008). Virus isolation has been performed as previously described (Spedicato et al., 2016).

2.4. Statistical analysis

The numbers of viraemic animals and that of rt-PCR_{BTV} positive animals in groups A, B, C, and K have been compared in each time post infection through the Friedman nonparametric test [40]. A post hoc analysis using the Nemenyi test with Bonferroni correction has been applied to assess the significance of two by two comparisons (Hollander and Wolfe, 1999). For this analysis, an animal was considered viraemic when it showed Log_{10} $TCID_{50}$ $ml^{-1} > 0$ and RNAemic when it showed a CT value <45 by rt-PCR_{BTV} in the blood samples. The effect of a second booster on humoral response has been evaluated in groups A and B. The SN titres before and after the booster in these groups have been compared through the non-parametric Wilcoxon test (Siegel and Castellan, 1956). The statistical analysis has been performed in XLStat (SOFTWARE: XlStat ver. 2013.2.04 – Copyright Addinsoft 1995–2013).

3. Results

None of the animals, including the non-vaccinated/infected animals (NVIA, Group K) showed relevant clinical signs of BTV infection during the experimental period, and rectal temperatures remained within the normal range for the species. We primarily investigated