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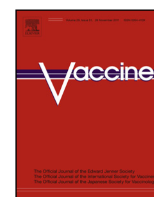

Inactivated Schmallenberg virus prototype vaccines

Kerstin Wernike [a,1], Veljko M. Nikolin [b,1], Silke Hechinger [a], Bernd Hoffmann [a], Martin Beer [a,*]

[a] Institute of Diagnostic Virology, Friedrich-Loeffler-Institut (FLI), Suedufer 10, 17493 Greifswald–Insel Riems, Germany
[b] Boehringer Ingelheim Veterinary Research Center GmbH & Co. KG, Bemeroder Str. 31, 30559 Hannover, Germany

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ABSTRACT

Schmallenberg virus (SBV), a novel *Orthobunyavirus*, is an insect-transmitted pathogen and was first described in Europe in 2011. SBV causes a mild transient disease in adult ruminants, but severe foetal malformation and stillbirth were observed after an infection of naive cows and ewes, which is responsible for considerable economic losses. The virus is now widely distributed in Europe, and no vaccines were available to stop transmission and spread.

In the present study, 16 calves and 25 sheep, the major target species of SBV infection, were vaccinated twice 3 weeks apart with one of 5 newly developed, inactivated vaccine candidates. Six calves and 5 sheep were kept as unvaccinated controls. All animals were clinically, serologically and virologically examined before and after challenge infection.

Immunisation with the inactivated preparations resulted in a neutralising antibody response three weeks after the second vaccination without any side effects. The number of animals that seroconverted in each group and the strength of the antibody response were dependent on the cell line used for virus growth and on the viral titre prior to inactivation. Four vaccine prototypes completely prevented RNAemia after challenge infection, a fifth candidate reduced RNAemia considerably. Although further evaluations e.g. regarding duration of immunity will be necessary, the newly developed vaccines are promising candidates for the prevention of SBV-infection and could be a valuable tool in SBV control strategies.

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1. Introduction

After the emergence of bluetongue virus serotype 8 (BTV-8) in 2006 [1], another vector-transmitted pathogen affecting domestic and wild ruminants was introduced to Northern Europe in 2011. The novel virus, referred to as “Schmallenberg virus” (SBV), was discovered near the German–Dutch border and thereafter spread rapidly to other European countries [2,3]. SBV is a member of the Simbu serogroup within the genus *Orthobunyavirus*; the most related viruses are Sathuperi virus and Douglas Virus [4]. Acute SBV infection causes no or only mild clinical signs including fever, reduced milk production or diarrhoea for a few days. However, an infection of naive cows and ewes during a critical period of pregnancy can lead to severe foetal malformations [5,6]; reviewed in [2,7].

Insect vectors are responsible for the spread of SBV and viral genome was detected in field-collected *Culicoides* spp. biting midges [8–10]. Besides other orthobunyaviruses, e.g. Oropouche virus or Akabane virus (AKAV), various members of the genus *Orbivirus* within the family *Reoviridae* have been isolated from *Culicoides* biting midges. As an example, bluetongue virus (BTV) is transmitted between its ruminant hosts mainly by the bites of midges [11]. European experience showed that vaccination of livestock against BTV has had a major role in reducing the virus circulation and even in eradicating the disease from some areas [12,13]. Based on similarities between BTV and SBV regarding involved insect vectors and affected host species the same is suspected for SBV. In addition, inactivated vaccines against AKAV and Aino virus, which are members of the Simbu serogroup within the genus *Orthobunyavirus*, can prevent the diseases [14]. In all likelihood, vaccination against SBV will be one of the most important aspects of disease control on the farm as well as on a region or country level. In the present study, several inactivated vaccine formulations have been produced and subsequently tested in sheep and cattle regarding their ability to induce neutralising antibodies and to prevent viraemia after experimental challenge infection.

2. Materials and methods

2.1. Vaccines

In order to test the immunogenicity of viruses grown on different cell substrates, five different prototype vaccine formulations

* Corresponding author. Tel.: +49 38351 71200; fax: +49 38351 71226.
E-mail address: martin.beer@fli.bund.de (M. Beer).
[1] These authors contributed equally to this work.

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Table 1
Vaccines and animal groups.

Vaccines			Inactivation	Animals	
Name	Cell line	Infectious titre used ($TCID_{50}$/ml)		Animal group	Animal number
BHKCT-HT	BHK-21 clone CT	5.7 log 10	Long protocol	A (sheep)	S01–S05
				G (cattle)	C01–C06
BHK13-HT	BHK-21 clone 13	5.7 log 10	Short protocol	B (sheep)	S06–S10
BHK13-LT	BHK-21 clone 13	4.7 log 10	Short protocol	C (sheep)	S11–S15
MA-HT	MA-104	6.1 log 10	Short protocol	D (sheep)	S16–S20
				H (cattle)	C07–C10
MA-LT	MA-104	5.7 log 10	Long protocol	E (sheep)	S21–S25
				I (cattle)	C11–C16
Unvaccinated control				F (sheep)	S26–S30
				K (cattle)	C17–C22

were produced (Table 1); all of them were inactivated SBV preparations in aqueous solution. SBV was either grown on two different baby hamster kidney (BHK-21) cell lines (vaccines "BHKCT-HT", "BHK13-HT", "BHK13-LT") or on MA-104 cells (vaccines "MA-HT" and "MA-LT"), a monkey kidney cell line. Both cell substrates, BHK-21 as well as MA-104 support the growth of the virus to high titers. The antigen was quantitated using the infectious titre of SBV before inactivation with binary ethylenimine (BEI), using either a long (using 10 mM of BEI for 72 h at 37 °C) or a short (using 2 mM of BEI for 12 h at 37 °C) protocol. Vaccine candidates contained antigen concentration as follows: 6.1 log 10 50% tissue culture infectious doses per ml ($TCID_{50}$/ml) (MA-HT) or 5.7 log 10 $TCID_{50}$/ml (BHKCT-HT, BHK13-HT, MA-LT) or 4.7 log 10 $TCID_{50}$/ml (BHK13-LT). Saponin (0.125 μg per 1 ml) and aluminium hydroxide (6.65 mg per ml) were used as adjuvants in all vaccine candidate formulations and the pH values were adjusted to pH 6.8–7.2 at 20 °C. All candidate vaccines were tested for the absence of bacterial contamination and for successful virus inactivation by two subsequent passages on BHK-21 cells. The vaccines were kept at 4 °C until use.

2.2. Animals

The experimental protocol was reviewed by a state ethics commission and has been approved by the competent authority (State Office for Agriculture, Food Safety and Fisheries of Mecklenburg-Vorpommern, Rostock, Germany, ref. LALLF M-V TSD/7221.3-1.1-004/12).

Twenty-five SBV-naive sheep of European domestic breeds (7–9 months of age) were assigned to 5 groups of 5 animals each, which were immunised subcutaneously with one of the prototype vaccines (see Table 1). Another 5 sheep were kept as unvaccinated controls. Male and female animals were distributed equally.

In addition, 22 SBV antibody-negative female Holstein–Friesian cattle were assigned to four groups of four (group H) or six animals (groups G, I and K) each. Animals in group G, H and I were immunised subcutaneously with vaccines BHKCT-HT, MA-HT and MA-LT, respectively. Cattle in group K were kept as unvaccinated controls. On the day of the first vaccination, the animals were between 8 and 12 months of age.

In each case, the animals were vaccinated twice three weeks apart and three weeks after the second vaccination both vaccinated and control animals were inoculated with 2 × 0.5 ml of an SBV field strain that was only passaged in the natural host [15]. During the entire study, rectal body temperatures were measured daily, and the animals were examined for clinical signs by veterinarians.

2.3. Sampling, real-time RT-PCR and serology

Following the first vaccination, serum samples were collected at days 0, 3, 4, 7 and weekly thereafter. After the second vaccination, serum samples were taken in weekly intervals. Following challenge infection, serum samples were taken daily during the first eight days and on days 14 and 21. Samples of spleen, tonsils, and mesenteric and mandibular lymph nodes were taken at autopsy on days 22–29 after challenge infection and homogenized in 1 ml MEM.

RNA from serum and tissue samples taken at autopsy was extracted using the MagAttract Virus Mini M48 Kit for automated extraction (Qiagen, Germany) according to the manufacturer's recommendations. SBV genome loads were determined by a reverse transcription real-time PCR (RT-qPCR) [16] with an external standard based on the S genome segment. Furthermore, serum samples were analyzed with a commercially available SBV antibody ELISA (ID Screen® Schmallenberg virus Indirect, IDvet, France) using the recommended cut-off of 70% relative optical density compared to the positive control, and in a standard micro-neutralisation assay as described previously [17].

3. Results

3.1. Clinical observations and post-mortem examinations

Following the first vaccination with the vaccine prototypes no adverse side effects were observed; none of the animals showed fever or any other clinical sign. After the second vaccination one cow immunised with vaccine MA-HT (group H) developed a low-grade swelling at the injection site for 2 days.

After the challenge infection, one unvaccinated cow developed fever on day 3, another one showed mild diarrhoea for three days. One animal from group I had nasal discharge for one day.

Autopsy of the animals did not reveal any significant gross lesions. The mesenteric lymph nodes of all but one (S30) unvaccinated animals were PCR-positive; on average 2.86E + 03 genome copies per mg (copies/mg) were detected. In addition, SBV RNA was found in the mandibular lymph nodes of 3 out of 5 unvaccinated sheep (S27–S29) and of all control cattle (average 2.68E + 01 copies/mg), the tonsils of S27–S29 and C18–C20 (average 9.90E + 01 copies/mg), and spleens of 4 out of 5 unvaccinated sheep (S26–S29; average 4.57E + 03 copies/mg) and of two control calves (C17, C21; average 1.40E + 01 copies/mg). No viral RNA was detected in any of the vaccinated animals.

3.1.1. Antibody response

On the day of the first vaccination, all animals were negative in both serological assays (data not shown). Before challenge infection, no antibodies could be detected in the unvaccinated animals. Three weeks after infection all but one (S30) control sheep and cattle scored positive in the neutralisation assay. Antibodies were found in cattle and in 2 out of the 5 unvaccinated sheep (S26, S29) by ELISA as well. Despite an increasing sample OD relative to the positive control OD value (S/P) both the control sheep S27 and S28 scored negative in the ELISA (Fig. 1).

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