Single immunization with an inactivated vaccine protects sheep from Schmallenberg virus infection
Silke Hechinger, Kerstin Wernike, Martin Beer

To cite this version:
Silke Hechinger, Kerstin Wernike, Martin Beer. Single immunization with an inactivated vaccine protects sheep from Schmallenberg virus infection. Veterinary Research, BioMed Central, 2014, 45 (1), pp.79. 10.1186/s13567-014-0079-6. hal-01290574

HAL Id: hal-01290574
https://hal.archives-ouvertes.fr/hal-01290574
Submitted on 18 Mar 2016

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Single immunization with an inactivated vaccine protects sheep from Schmallenberg virus infection

Silke Hechinger, Kerstin Wernike and Martin Beer*

Abstract

The arthropod-borne Schmallenberg virus (SBV), family Orthobunyaviridae, emerged in Europe in 2011. SBV is associated with a mild disease in adult ruminants but fetal malformation after an infection during a critical phase of pregnancy. A number of inactivated vaccines have been developed; their efficacy after two injections was demonstrated. To make the vaccination of sheep more efficient and economic the effect of a single immunization with one of these vaccines was investigated in the present study. Five vaccinated sheep and five additional control sheep were inoculated with SBV three weeks after vaccination and the results of a competitive ELISA, a standard microneutralization test and an SBV-specific real-time RT-PCR confirmed vaccine efficacy by demonstrating complete inhibition of viral replication in immunized animals.

Introduction, methods and results

A previously unknown pathogen from the family Orthobunyaviridae emerged in Europe in autumn 2011 and was named Schmallenberg virus (SBV) according to the location of its discovery [1]. Midges (Culicoides spp.) are involved in its transmission [2-4]. While disease was first observed in cattle, sheep and goats, infection has also been detected in deer, bison, alpaca, moose and other wild ruminants [5]. The clinical picture is characterized by mild febrile disease in adult ruminants and the potential development of fetal malformations after transplacental infection [6-9]. An SBV-infection can be confirmed through detection of viral RNA both in serum during the first week post infection and in tissue samples [10].

As an effective instrument for disease control different inactivated vaccines have been developed and tested [11]. Besides, two commercial inactivated vaccines have already been granted a provisional marketing authorization in the United Kingdom and France, respectively [12,13].

Until now, only studies about a protective effect after two vaccinations have been published. Reduction to a single injection minimizes workload and costs, which is especially important for sheep owners, as the animals are usually individually caught and restrained on the pastures for every injection. Therefore, the influence of a single immunization on a subsequent SBV-inoculation of sheep was investigated in the present study.

Five SBV-negative yearling sheep (S01 to S05) of European domestic breeds received a single subcutaneous injection with 2 mL of the MA-HT prototype vaccine from a previous study [11]. Five additional control sheep (S06 to S10) were left unvaccinated.

Three weeks after vaccination all animals were inoculated with $2 \times 0.5$ mL of calf serum containing an SBV field strain that was only passaged in the natural host. The production of this infectious serum has been described earlier [10]. The serological status was monitored weekly by a blocking ELISA (ID Screen® Schmallenberg virus Competition, ID vet, France) and a standard microneutralization test (SNT) [14]. Additionally, blood samples were taken daily on the 8 days following challenge infection and tested by ELISA and an SBV-specific reverse transcription real-time PCR (RT-qPCR) including an external standard based on the small (S) genome segment [15]. Rectal body temperatures were recorded daily during the entire study and the animals were examined daily for clinical signs. Autopsy was conducted three weeks after challenge infection and samples of spleen, mesenteric and mandibular
lymph node and tonsils were taken and tested by RT-qPCR.

According to German legislation 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. No. LALLF M-V TSD/7221.3-1.1-004/12).

The SBV-antibody-ELISA was used according to the manufacturer’s instructions. Results were calculated as the ratio of the optical density (OD) of the sample and the OD of the negative control (S/N, %). Samples with an S/N-value of 40% or less were considered positive. ELISA results are calculated as the ratio of the optical density (OD) of the sample and the OD of the negative control (S/N, %).

Neutralizing titers (SNT) are given as the reciprocal of the serum dilution showing 50% virus neutralization. Titers of 5 or more were considered positive.

All vaccinated animals were positive for SBV prior to challenge infection in at least one serological test (Table 1) and, thereafter, neutralizing titers remained largely constant with values between 7 and 14 ND50 on day 21 post challenge. The first antibodies were detected by SNT in S02 on day 7 after vaccination while the ELISA gave negative results for this animal for all sampling dates except day 14 after vaccination (Table 1). Samples of S01, S03 and S05 gave positive results in the SNT starting from day 14 after vaccination while S01 and S03 scored negative in the ELISA throughout the study. S05 gave only one doubtful ELISA result on day 14 post vaccination. In S04 neutralizing antibodies were detectable only one week after challenge infection but it scored positive in the ELISA on day 14 after vaccination and doubtful on the day of challenge infection.

After challenge infection, SBV-RNA was detectable in serum samples of all control animals for at least 3 consecutive days (Table 2). The mean maximum genome load in serum samples was $9.2 \times 10^4$ genome copies per mL. Most tissue samples of the control animals gave positive PCR results as well. Only tonsils and mesenteric lymph nodes of S10, and mandibular lymph nodes of S06 scored negative. Mean genome loads per gram organ weight were $1.2 \times 10^4$ copies/g for mandibular lymph nodes (minimum value: $4.9 \times 10^2$ copies/g; maximum value: $3.6 \times 10^5$ copies/g), $8.7 \times 10^4$ copies/g for mesenteric lymph nodes (min: $5.9 \times 10^1$; max: $3.4 \times 10^5$), $1.2 \times 10^5$ copies/g for spleens (min: $6.8 \times 10^3$; max: $3.9 \times 10^3$) and $1.6 \times 10^5$ copies/g for tonsils (min: $4.7 \times 10^3$; max: $6.0 \times 10^3$).

In contrast, viral RNA was not detected in any serum or tissue sample from the vaccinated animals.

### Table 1 Serological results

| Animal | Group | SNT<sup>a</sup> | ELISA<sup>a</sup> | qRT-PCR |
|--------|-------|----------------|------------------|---------|
|        |       | 0 dpv 14 dpv 0 dpv 7 dpv 14 dpv 21 dpv | 0 dpv 14 dpv 0 dpv 7 dpv 14 dpv 21 dpv tissue |
| S01    | vac   | < 5 < 5 < 5 < 5 < 5 < 5 | 17 12 10 10 10 7 | 91.6 51.5 53.4 59.6 56.5 53.5 - |
| S02    | vac   | < 5 < 5 < 5 < 5 < 5 < 5 | 56 24 14 10 14 14 | 92.0 45.8 54.9 55.1 65.6 70.2 - |
| S03    | vac   | < 5 < 5 < 5 < 5 < 5 < 5 | 12 7 6 7 14 14 | 97.1 50.8 60.1 64.7 51.3 50.1 - |
| S04    | vac   | < 5 < 5 < 5 < 5 < 5 < 5 | 7 7 7 7 7 7 | 84.4 33.0 42.4 46.0 51.8 56.6 - |
| S05    | vac   | < 5 < 5 < 5 < 5 < 5 < 5 | 20 28 14 12 12 12 | 92.8 47.5 54.7 57.3 54.6 88.4 - |
| S06    | co ND ND < 5 < 5 < 5 < 5 | 14 67 40 40 40 40 | ND ND 92.5 42.5 26.9 26.5 + |
| S07    | co    | < 5 < 5 < 5 < 5 < 5 < 5 | 12 375 266 266 266 266 | 94.9 89.6 94.8 43.8 14.6 17.5 + |
| S08    | co    | < 5 < 5 < 5 < 5 < 5 < 5 | 8 160 67 67 67 67 | 95.9 86.7 92.0 30.2 30.6 37.9 + |
| S09    | co    | < 5 < 5 < 5 < 5 < 5 < 5 | 10 224 160 160 160 160 | 99.6 91.0 97.3 44.5 23.1 21.9 + |
| S10    | co    | < 5 < 5 < 5 < 5 < 5 < 5 | 8 56 33 33 33 33 | 82.4 67.9 73.9 58.8 32.3 27.6 + |

Key serological results for vaccinated (vac) and control (co) animals are presented. Serological results are given for 0 and 14 days post vaccination (dpv) to 21 days post challenge infection (dpc). Neutralizing titers (SNT) are given as the reciprocal of the serum dilution showing 50% virus neutralization. Titers of 5 or more were considered positive. ELISA results are calculated as the ratio of the optical density (OD) of the sample and the OD of the negative control (S/N, %). Samples with an S/N value of 40% or less are considered positive. Results for RNA detection are given for tissue samples obtained at autopsy.

<sup>a</sup>Positive or doubtful ELISA results and positive SNT results are highlighted by bold figures.
Discussion
In the unvaccinated control animals PCR results demonstrated viral replication and dissemination. The serological results support this observation as the SBV-infection induced a pronounced humoral immune response. In all vaccinated sheep, on the other hand, the absence of RNAemia demonstrates the protective effect of immunization. Furthermore, the antibody titers remained constant which suggests that the virus is eliminated before a memory immune response with an antibody boost could be triggered. The latter is in accordance with results of an earlier study in cattle [14] during which constant neutralizing titers were detected in seropositive animals after a second experimental SBV-infection. Interestingly, the single shot vaccination was highly efficacious and could even prevent both viremia and infection of target tissues such as mesenteric lymphnodes.

Interestingly, neutralizing titers are very low in vaccinated animals in this study and only a few ELISA results of their serum samples exceed the cut-off value. Thus, further factors, e.g. a cellular immune response, may contribute to the protective effect of vaccination. Similar observations have been reported for Rift Valley Fever virus (RVFV, family Bunyaviridae, genus Phlebovirus). Neutralizing antibodies are primarily responsible for protection against RVFV-infection [16]. However, one of six lambs treated with an inactivated vaccine showed a reduction in viremia and lack of clinical symptoms although detectable neutralizing antibodies were missing at the time of infection [17]. Furthermore, a study on Crimean-Congo hemorrhagic fever virus (CCHFV, family Bunyaviridae, genus Nairovirus) reports that an inactivated vaccine is able to elicit a considerable T-cell reaction in humans as measured by IFN-gamma production [18].

Unfortunately, there are no immunological studies available which deal with orthobunyavirus vaccines. Thus, the exact mechanism underlying our observations remains unclear. However, as saponins are able to stimulate cellular immune responses [19], this adjuvant used for the formulation of the vaccine may be an important factor for vaccine efficacy and protection from SBV-replication post challenge infection.

In conclusion, the present study demonstrated the complete protection of sheep from SBV-infection after a single injection while the underlying immunological mechanism needs to be further investigated.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
Conceived and designed the experiments: KW, MB. Performed the experiments: SH, KW. Analyzed the data: SH, KW. Wrote the paper: SH, KW, MB. All authors read and approved the final manuscript.

Acknowledgements
We thank Anja Landmesser for outstanding technical assistance. Dedicated animal care was provided by the staff of the quarantine and BSL-3 facilities of the Friedrich-Loeffler-Institut. This work was financially supported by Boehringer Ingelheim Vetmedica GmbH.

Received: 20 May 2014 Accepted: 21 July 2014 Published: 3 August 2014

Table 2 RNA detection in serum post challenge

| Animal | Group | Number of SBV-RNA copies per mL serum |
|--------|-------|----------------------------------------|
|        | 0 dpc | 1 dpc | 2 dpc | 3 dpc | 4 dpc | 5 dpc | 6 dpc | 7 dpc | 8 dpc |
| S 06   | co    | -     | -     | 4.5 × 10^3 | 3.9 × 10^3 | 2.2 × 10^3 | ND     | -     | -     |
| S 07   | co    | -     | -     | 1.9 × 10^3 | 1.2 × 10^3 | 7.0 × 10^3 | ND     | 1.4 × 10^3 | -     |
| S 08   | co    | -     | -     | 1.4 × 10^3 | 1.5 × 10^4 | 2.5 × 10^4 | ND     | -     | -     |
| S 09   | co    | -     | -     | 5.2 × 10^3 | 3.3 × 10^4 | 1.5 × 10^4 | ND     | -     | -     |
| S 10   | co    | -     | -     | 8.5 × 10^3 | 1.8 × 10^3 | 6.4 × 10^3 | ND     | -     | -     |

Results are given for 0 to 8 days post challenge (dpc) for control animals (co). Dashes represent negative PCR results of the respective samples. Viral RNA was not detected in the serum of vaccinated animals at any time. Therefore, vaccinated animals were not included in the table. From 5 dpc serum samples were not available. Consequently, the RNA load could not be determined (ND) for this time point.

References
1. Hoffmann B, Scheuch M, Höper D, Jungblut R, Holsteg M, Schirmeier H, Eschbaumer M, Goller K, Werneke K, Fischer M, Breithaupt A, Mettenleiter TC, Beer M. Novel orthobunyavirus in Cattle, Europe, 2011. Emerg Infect Dis 2012, 18:469–472.
2. De Regge N, Deblauwe I, De Deken R, Vantieghem P, Madder M, Geysen D. Detection of Schmallenberg virus in different Culicoides spp. by real-time RT-PCR. Transbound Emerg Dis 2012, 59:471–475.
3. Elbers AR, Meuwissen R, van Weezep E, Sloet van Oldruitenborgh-Oosterbaan MM, Kooi EA. Schmallenberg virus in Culicoides spp. biting midges, the Netherlands, 2011. Emerg Infect Dis 2013, 19:106–109.
4. Rasmussen LD, Kristensen B, Kirkeby C, Rasmussen TB, Beltham GJ, Bäder K, Bäärner A. Culicoides as vectors of schmallenberg virus. Emerg Infect Dis 2012, 18:1204–1206.
5. European Food Safety Authority. “Schmallenberg” virus: analysis of the epidemiological data. (May 2013) Supporting Publications 2013:EN-429 [22 pp.]; available online [http://www.efsa.europa.eu/en/search/doc/429e.pdf]; (last accessed: May 2014).
6. Garigliani MM, Hoffmann B, Dive M, Sarfetel A, Boyrou C, Cassign C, Beer M, Desmecht D. Schmallenberg virus in calf born at term with porencephaly, Belgium. Emerg Infect Dis 2012, 18:1005–1006.
7. Herder V, Wohlsein P, Peters M, Hansmann F, Baumann W. Salient lesions in domestic ruminants infected with the emerging so-called Schmallenberg virus in Germany. Vet Pathol 2012, 49:588–591.
8. Martinelle L, Dal Pozzo F, Gauthier B, Kirschvink N, Saegerman C. Field veterinary survey on clinical and economic impact of Schmallenberg virus in Belgium. Transbound Emerg Dis 2014, 61:285–288.
9. van den Brom R, Luttikholt SJ, Lievaart-Peterson K, Peperkamp NH, Mars MH, van der Poel WH, Vellema P: Epizootic of ovine congenital malformations associated with Schmallenberg virus infection. Tijdschr Diergeneesk 2012, 137:106–111.

10. Wernike K, Eschbaumer M, Breithaupt A, Hoffmann B, Beer M: Schmallenberg virus challenge models in cattle: infectious serum or culture-grown virus? Vet Res 2012, 43:84.

11. Wernike K, Nikolin VM, Hechinger S, Hoffmann B, Beer M: Inactivated Schmallenberg virus prototype vaccines. Vaccine 2013, 31:3558–3563.

12. Merck Animal Health. [http://www.merck-animal-health.com/news/2013-5-21.aspx] (last accessed: May 2014).

13. Merial. [http://fc.rmp.merial.com/SitePages/view_RCP_notice.aspx?NomProduit=SRVAX] (last accessed: May 2014).

14. Wernike K, Eschbaumer M, Schirmeier H, Blohm U, Breithaupt A, Hoffmann B, Beer M: Oral exposure, reinfection and cellular immunity to Schmallenberg virus in cattle. Vet Microbiol 2013, 165:155–159.

15. Bilk S, Schulze C, Fischer M, Beer M, Hlinak A, Hoffmann B: Organ distribution of Schmallenberg virus RNA in malformed newborns. Vet Microbiol 2012, 159:236–238.

16. Ikegami T, Makino S: Rift valley fever vaccines. Vaccine 2009, 27(Suppl 4):D69–D72.

17. Kortekaas J, Antonis AF, Kant J, Vloe V, Vogel A, Oreshkova N, de Boer SM, Bosch BJ, Moormann RJ: Efficacy of three candidate Rift Valley fever vaccines in sheep. Vaccine 2012, 30:3423–3429.

18. Mousavi-Jazi M, Karlberg H, Papa A, Christova I, Mirazimi A: Healthy individuals’ immune response to the Bulgarian Crimean-Congo hemorrhagic fever virus vaccine. Vaccine 2012, 30:6225–6229.

19. Song X, Hu S: Adjuvant activities of saponins from traditional Chinese medicinal herbs. Vaccine 2009, 27:4883–4890.

doi:10.1186/s13567-014-0079-6
Cite this article as: Hechinger et al.: Single immunization with an inactivated vaccine protects sheep from Schmallenberg virus infection. Veterinary Research 2014 45:79.

Submit your next manuscript to BioMed Central and take full advantage of:

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at www.biomedcentral.com/submit