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# Rapid spread and association of Schmallenberg virus with ruminant abortions and foetal death in Austria in 2012/2013

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ABSTRACT

Schmallenberg virus (SBV) has emerged in summer–autumn 2011 in north-western Europe. Since then, SBV has been continuously spreading over Europe, including Austria, where antibodies to SBV, as well as SBV genome, were first detected in autumn 2012. This study was performed to demonstrate the dynamics of SBV spread within Austria, after its probable first introduction in summer 2012. True seroprevalence estimates for cattle and small ruminates were calculated to demonstrate temporal and regional differences of infection. Furthermore, the probability of SBV genome detection in foetal tissues of aborted or stillborn cattle and small ruminants as well as in allantoic fluid samples from cows with early foetal losses was retrospectively assessed.

SBV first reached Austria most likely in July–August 2012, as indicated by retrospective detection of SBV antibodies and SBV genome in archived samples. From August to October 2012, a rapid increase in seroprevalence to over 98% in cattle and a contemporaneous peak in the detection of SBV genome in foetal tissues and allantoic fluid samples was noted, indicating widespread acute infections. Notably, foetal malformations were absent in RT-qPCR positive foetuses at this time of the epidemic. SBV spread within Austrian cattle reached a plateau phase as early as October 2012, without significant regional differences in SBV seroprevalence (98.4–100%). Estimated true seroprevalences among small ruminates were comparatively lower than in cattle and regionally different (58.3–95.6% in October 2012), potentially indicating an eastward spread of the infection, as well as different infection dynamics between cattle and small ruminants. Additionally, the probability of SBV genome detection over time differed significantly between small ruminant and cattle samples subjected to RT-qPCR testing.



## 1. Introduction

Schmallenberg virus (SBV), an orthobunyavirus of the Simbu serogroup, has emerged in summer–autumn 2011 in north-western Germany and the Netherlands and has since spread over most of Europe (Hoffmann et al., 2012; Beer et al., 2013; EFSA, 2013).

According to current knowledge, the virus is mainly – if not exclusively – spread by blood-sucking arthropods belonging to the genus *Culicoides* (Rasmussen et al., 2012; Elbers et al., 2013a,b; Goffredo et al., 2013). SBV is able to infect several species of ruminants and New World

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camelids, including cattle, sheep, goats, bison, buffalo, red deer, fallow deer, roe deer, moose, mouflon, chamois and alpaca (Linden et al., 2012; EFSA, 2012, 2013; Schiefer et al., 2013a). In addition, SBV antibodies (SBV-AB) have recently been described in a dog (Wensman et al., 2013).

Post-partum, SBV infection is subclinical, or leads to transient, clinically unspecific symptoms like fever, diarrhoea and milk loss (Hoffmann et al., 2012; Wernike et al., 2013a,b).

Serological studies performed in countries that were already affected by SBV infections in 2011 reported animal and within-herd seroprevalences, respectively, in the range of 63–99% in cattle and 84–89% in sheep, as well as between-herd seroprevalences that were close to 100% in both species. This indicates a very efficient and rapid spread of the infection in 2011 (Elbers et al., 2012; Méroc et al., 2013a,b; Veldhuis et al., 2013).

In immunologically naïve pregnant cows, sheep and goats, the virus may cross the placenta and infect the foetus, leading to damage of the developing nervous system. As a result, diverse foetal cerebrospinal as well as musculoskeletal malformations may occurr (Herder et al., 2012; Hahn et al., 2013); these are usually summarized under the term arthrogryposis-hydranencephaly-syndrome (AHS) and are typically observed around term, months after the actual infection event. Congenital malformations confirmed by detection of SBV genome were reported from a maximum of 4% of sheep holdings and less than 1.3% of cattle holdings per country. Most confirmed foetal malformations were reported in spring 2012, with an observable time difference in peak reporting frequency between small ruminants (sheep and goats) and cattle (EFSA, 2012). In contrast, little is currently known about potential immediate effects of SBV infection on the foetus, such as the occurrence and frequency of abortion as immediate consequence of infection. This is mainly due to SBV being unknown at the time of the first SBV expansion in north-western Europe; thus, abortions occurring in summer–autumn 2011 were not related to SBV, and retrospective studies on archived tissues have not been published.

In response to the SBV epidemic in north-western Europe, Austria performed a national SBV monitoring programme on cattle and small ruminant samples (~1200) collected from October 2011 to June 2012; all sera from Austrian animals tested negative for SBV AB, indicating that Austria was indeed not affected by the first expansion of SBV in 2011 (Schiefer et al., 2013b). This active monitoring programme was discontinued by the end of June 2012 and replaced by passive surveillance. Soon after that, detection of acute SBV infections in the neighbouring German federal states of Baden-Württemberg and Bavaria as well as in Switzerland in summer–autumn 2012 (Conraths et al., 2013; Schorer et al., 2012) suggested that SBV introduction to Austrian territory was probably soon to follow. Indeed, SBV-AB were first detected in Austrian cattle and sheep (proMED-mail, 2012a) in September 2012, closely followed by confirmation of SBV genome detection in bovine foetal tissue on October 1st, 2012.

The aim of this study was to analyze the dynamics of the SBV epidemic in Austrian cattle and small ruminants by performing a spatio-temporal analysis of SBV spread within the country. For that purpose, cattle, sheep and goat blood samples that were collected in the course of the epidemic were tested for SBV-AB.

Regional seroprevalences in both, cattle and small ruminants, were estimated on a monthly basis. Multivariate modelling was applied in order to identify variables with potentially significant influence on SBV-AB status, such as sampling time, region, age, sex and host species (in small ruminants), as well as (in cattle only) farm type, breed, herd size and cattle density. Serological testing also involved a small number of samples collected in autumn–winter 2012 from wild ruminant species and New World camelids.

To investigate the impact of the SBV epidemic on ruminant reproduction, all cattle and small ruminant abortions received from August 2012 to July 2013 were tested for the presence of SBV genome, irrespective of the presence of AHS-type malformations. Furthermore, allantoic fluid or foetal tissue collected after diagnosis of early foetal death in cattle was also tested for SBV genome.

## 2. Materials and methods

### 2.1. Cattle, sheep, goat, New World camelid and wild ruminant blood samples

A total of 3359 blood samples from cattle, 1031 samples from sheep and 230 goat samples collected from July to December 2012 were tested for SBV-AB. Of cattle samples, 1247 samples were collected in the course of the national Bluetongue monitoring programme, 1125 were from the national screening programme for Bovine brucellosis, Enzootic bovine leucosis and Infectious bovine rhinotracheitis, 798 were tested for SBV-AB on private commission, 155 blood samples were obtained accompanying abortions and 34 samples could not be assigned to any of these categories. Of sheep and goat samples, 568 (209) were from the national *Brucella melitensis* screening programme, 368 (0) were tested for SBV-AB on private commission, 28 (0) were accompanying abortions and 67 (21) could not be assigned to any of these categories.

New World camelid and wild ruminant blood samples were collected from October 2012 to January 2013 (n = 111) and originated from animals harvested from the wild, or kept in farms or zoos.

Sera were harvested from whole blood after centrifugation at 1600 g for 5 min and either tested directly, or stored at −20 °C until testing.

### 2.2. Serological testing

Cattle, sheep, goat, wild ruminant and New World camelid sera were tested with the ID Screen® Schmallenberg Virus Indirect ELISA (ID.vet, Montpellier, France). Assay setup and interpretation strictly followed the manufacturer's instructions. According to information from the manufacturer, the diagnostic sensitivity and specificity of the ELISA is 97.72% and 99.67%, respectively.