Preventive Veterinary Medicine 112 (2013) 35–47

Contents lists available at SciVerse ScienceDirect

Preventive Veterinary Medicine

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

Schmallenberg virus epidemic in the Netherlands: Spatiotemporal introduction in 2011 and seroprevalence in ruminants

A.M.B. Veldhuis[a,*], G. van Schaik[a], P. Vellema[a], A.R.W. Elbers[b], R. Bouwstra[b], H.M.J.F. van der Heijden[a], M.H. Mars[a]

[a] GD Animal Health Service, Deventer, The Netherlands
[b] Central Veterinary Institute, Part of Wageningen UR, Lelystad, The Netherlands

ARTICLE INFO

Article history:
Received 22 December 2012
Received in revised form 12 June 2013
Accepted 23 June 2013

Keywords:
Schmallenberg virus
Seroprevalence
Introduction
Sheep
Goats
Cattle
The Netherlands

ABSTRACT

This study aimed at estimating the Schmallenberg virus (SBV) seroprevalence in dairy heifers, non-dairy adult cattle, sheep and goats in the Netherlands after cessation of SBV transmission at the end of 2011. Archived serum samples from ruminants submitted to the GD Animal Health Service for monitoring purposes between November 2011 and March 2012 were selected and tested for presence of SBV-specific antibodies using an in-house ELISA. Animal seroprevalences were estimated at 63.4% in dairy heifers, 98.5% in adult non-dairy cattle, 89.0% in sheep and 50.8% in goats. Multivariable analyses were carried out to describe the relationship between potential risk factors and the ELISA outcome S/P%. The overall SBV seroprevalence in ruminants and ruminant herds in the Netherlands at the end of 2011 was high, with considerable differences between species and farm types. No gradient spatial pattern in final seroprevalence could be detected and therefore no suggestions about the site of introduction and spread of SBV in the Netherlands in 2011 could be made. In dairy heifers, it was shown that S/P% increased with age. In sheep, S/P% was lower in animals located in the coastal area. Whether herds were located near the German border did not affect the S/P% in sheep nor in dairy heifers. An attempt was made to gain insight in the spatiotemporal introduction of SBV in the Netherlands in 2011, by testing sheep serum samples from 2011. A seroprevalence of about 2% was found in samples from April, June and July 2011, but the ELISA positive samples could not be confirmed in a virus neutralization test. A clear increase in seroprevalence started at August 2011. From mid-August 2011 onwards, seropositive samples were confirmed positive by virus neutralization testing. This indicated the start of the epidemic, but without a clear spatial pattern.

1. Introduction

In the fall of 2011, a new orthobunyavirus was identified in dairy cows with fever, drop in milk production and diarrhea, especially in the eastern regions of the Netherlands (Muskens et al., 2012) and in cows with milk drop in northwestern Germany (Hoffmann et al., 2012). The virus was tentatively named Schmallenberg virus (SBV) and belongs to the Simbu serogroup of the genus *Orthobunyavirus* of the family *Orthobunyaviridae* (Hoffmann et al., 2012). The S and L RNA segment of SBV has a high similarity with those of Shamonda virus, while the M RNA segment of SBV is closely related to the M segment of Sathuperi virus (Yanase et al., 2012; Goller et al., 2012). To our knowledge, clinical signs of these viruses have never been detected in Western-Europe in the past. From November 2011 on, SBV was

* Corresponding author at: GD Animal Health Service, Epidemiology Group att. Anouk Veldhuis, P.O. Box 9, 7400 AA Deventer, The Netherlands. Tel.: +31 570660408; fax: +31 570660354.
E-mail addresses: a.veldhuis@gddeventer.com, anoukveldhuis@hotmail.com (A.M.B. Veldhuis).

0167-5877/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.prevetmed.2013.06.010

found to cause an outbreak of congenital malformations in lambs and goat kids (Van den Brom et al., 2012a) and newborn calves (Hoffmann et al., 2012). Orthobunyaviridae are generally transmitted by arthropods. SBV has now been detected in several different *Culicoides* species in different parts of Europe in 2011 (Rasmussen et al., 2012; De Regge et al., 2012; Elbers et al., 2013). The first detection of clinical signs in the eastern regions of the Netherlands and northwestern Germany suggests that the epidemic started in the border area between Germany and the Netherlands. In the Netherlands, clinical signs of SBV infection were notifiable between December 20th, 2011 and July 6th, 2012, i.e. malformations in newborn ruminants had to be reported to the authorities. To detect antibodies in blood samples, a virus neutralization test (VNT) has been developed (Loeffen et al., 2012), which has been used for a first seroprevalence estimate in dairy cattle (Elbers et al., 2012). However, to gain more insight in the level of SBV infection in both ruminants (dairy and non-dairy) and small ruminants in the Netherlands, as well as the geographic spread, a large-scale seroprevalence study was needed. For this purpose, a SBV-ELISA is more practical and cost-effective. To date, SBV infections have been reported in many European countries. However, few results of seroprevalence studies providing the actual level of infection in these countries so far are published. In Belgium, within-herd seroprevalences in cattle, sheep and goats were estimated at 86.3% (Méroc et al., 2013a), 84.3% and 40.7%, respectively (Méroc et al., 2013b). In Germany, a median within-herd seroprevalence of 36.7% was found in goats (Helmer et al., 2013). The primary objective of the current study was to determine SBV seroprevalence in the Netherlands (by means of an SBV-ELISA) to gain insight in the true rate of infection in cattle, sheep and goats after cessation of virus circulation in 2011. By doing so, a better understanding of the potential damage following overwintering of SBV into a new vector-active season, indicated by the (remaining) proportion of naïve animals, was established. The second objective was to identify risk factors for SBV infection, in terms of the magnitude of antibody responses (sample to positive ratio; S/P%). Finally, an attempt was made to identify the spatiotemporal introduction of this new virus in the Netherlands by determining the pattern of seroprevalence in archived sheep serum samples.

2. Materials and methods

2.1. Seroprevalence

2.1.1. Study population in the Netherlands

In 2011, the cattle population in the Netherlands comprised 18,589 dairy herds and 18,431 non-dairy herds. In these herds, at least one animal was present in 2011 according to the national identification and registration (I&R) database. Most dairy herds can be found in the northern and eastern region of the Netherlands. Most non-dairy (i.e. meat-producing herds, suckling herds and traders) are located in the eastern part of the Netherlands. In 2011, approximately 27,000 (dairy and/or meat producing) sheep holdings were active according to the I&R database, of which 31% can be considered professional sheep holding. At least 30 animals is generally applied to distinguish professional small-ruminant holdings from hobbyists in the Netherlands, based on the frequency distribution of small ruminant herd sizes at national level. Sheep density is highest in the northern and northwestern regions of the Netherlands. Approximately 10,000 goat holdings were active in the Netherlands in 2011, of which about 5% can be considered professional (i.e. large-scale with >30 goats). The highest (dairy) goat density can be found in the southeastern part of the Netherlands.

2.1.2. Sampling design

Archived serum samples from ruminants submitted to the Animal Health Service (AHS) for monitoring purposes between November 2011 and March 2012 were selected. It was assumed that SBV virus circulation was very limited or had ceased in this period as a result of reduced activity of the vector. Thus, a reliable estimate of the seroprevalence at the end of the 2011 epidemic could be made. In order to estimate SBV-specific seroprevalences in the Dutch ruminant population, serum samples from dairy heifers, adult non-dairy cattle, and adult sheep and goats were selected. Table 1 provides an overview of the selected samples and their origin. All serum samples were stored at −20 °C at the AHS. Sample sizes were calculated using WIN EPISCOPE 2.0 (De Blas et al., 2000), assuming perfect test sensitivity and specificity.

Samples were investigated for presence of antibodies against SBV by means of an in-house indirect whole virus ELISA (Van der Heijden et al., 2013) with a sensitivity of 98.8% (95% confidence interval (CI): 93.3–99.8) and a specificity of 98.8% (95% CI: 97.5–99.6). Test outcomes were expressed as a sample to positive percentage (S/P%) by comparing the net optical density (OD) of each sample with the average net OD of positive controls. Test outcomes were considered to be non-specific when the serum sample reacted with the control antigen (without viral antigen) resulting in an OD greater than 1.0, irrespective of the sample's gross optical density. Samples with an S/P% higher than 15% were considered positive. This cut-off was determined using a SBV virus neutralization test (Loeffen et al., 2012) as golden standard. Only samples containing sufficient serum to perform ELISA testing were used. Seroprevalences were estimated on both animal and herd level (i.e. the herd is considered seropositive when $\geq$1 sample tested positive).

SBV seroprevalence estimation for non-dairy adult cattle was performed based on serum samples that were originally collected to estimate Infectious Bovine Rhinotracheitis (IBR) seroprevalence in the Netherlands. For that purpose, 470 non-dairy herds were randomly selected, of which a maximum of 30 samples per herd were collected between September 30th, 2011 and January 11th, 2012. Only herds without an IBR-free status (approximately 87% of the non-dairy herds), by participating in the voluntary IBR monitoring program in 2011, were originally considered for selection. Based on the assumption that most of the SBV transmission had taken place before November 1st, 2011, only serum samples collected after October 31st 2011 were included in the current study. Focusing on regional differences, a maximum of 150 sera