

## SCHMALLENBERG VIRUS – A NEW RISK IN CATTLE BREEDING IN EUROPE\*

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### Abstract

Since August 2011 Europe has been facing a new virus which attacks the domestic and wild ruminants. The virus was named after the town where the first isolation had been made. The virus in question is transmitted by the biting midges (*Culicoides* spp.) and it can survive the winter in the bodies of those insects. It is also known that the virus does not endanger human health and it cannot be transferred directly from one animal to another because it is only carried by the vectors.

**Key words:** cattle, malformations, ruminants, Schmallenberg virus

In August 2011 a previously unknown disease of dairy cattle was reported. It first appeared in Germany. The non-specific symptoms were mainly: a decreased milk production, a watery diarrhoea and a high fever in adult dairy cows. The symptoms disappeared after a few days (Hoffman et al., 2012). However some congenital malformations were later discovered in unborn lambs, calves and kids (Herder et al., 2012; Van den Brom et al., 2012). All previously known disease entities specific to the ruminants were excluded during the research in Friedrich Loeffler Institute in Germany, where a metagenomic analysis was conducted on the blood samples of a set of clinically ill dairy cows. This analysis led to detection of a new virus which belongs to the Simbu serogroup of the genus *Orthobunyavirus* of the family *Bunyaviridae* (Hoffmann et al., 2012). The new virus was named Schmallenberg virus (SBV) after the place where the first positive samples were collected.

According to the EFSA report (Anonymous, 2013) as many as 22 European countries reported an SBV infection and Poland was also among them. During the period September 2011 to April 2013 there were more than 8000 farms with laboratory con-

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firmed cases of SBV in Europe. Figure 1 shows European countries which reported cases of SBV and those countries where the viruses have not been found yet.

The aim of this review article is to look at the new disease entity caused by SBV, determine its origin and its way of spreading.

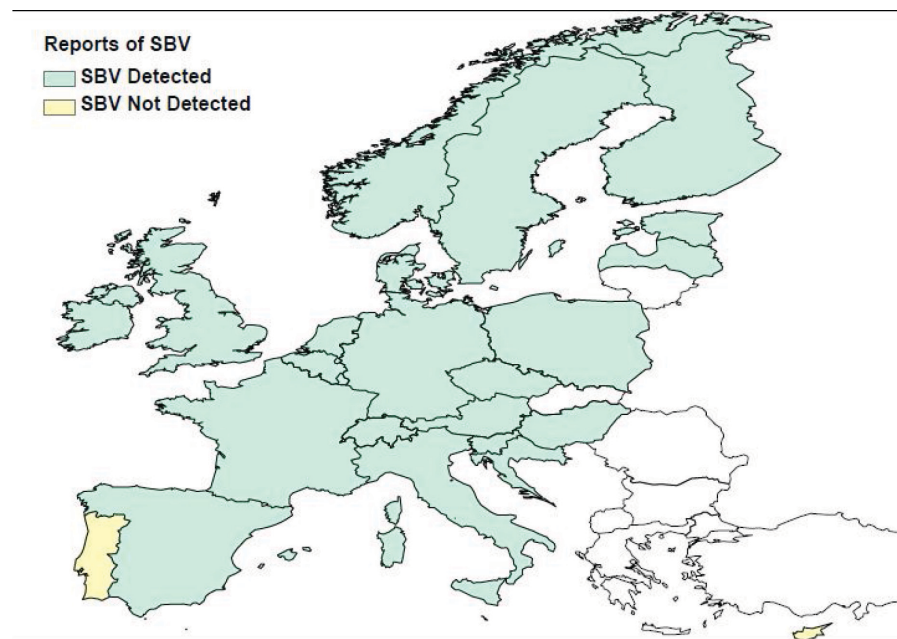


Figure 1. SBV status for European countries (Anonymous, 2013)

### Taxonomy of virus

The viruses of the genus *Orthobunyavirus* often occur in Asia, Africa and Oceania. They are included in the group of arboviruses which are normally transmitted by insects. Especially the Simbu serogroup, which includes Akabane, Aino and Shamoda viruses, can act as pathogens in ruminants. However, the viruses of this serogroup have not previously been detected in Europe (Hoffmann et al., 2012). It is certain that the biting midges (*Culicoides* spp.) are responsible for transmitting SBV (Bilk et al., 2012; Elbers et al., 2013; Rasmussen et al., 2012) and the ruminants, both domestic and wild ones are sensitive to the infection.

Family: *Bunyaviridae*,

Genus: *Orthobunyaviruses*,

Putative serogroup: *Simbu serogroup*,

Virus: Schmallenberg virus (Hoffmann et al., 2012).

The viruses in the family *Bunyaviridae* are classified into five genera: *Orthobunyavirus*, *Hantavirus*, *Nairovirus*, *Phlebovirus* and *Tospovirus*. The *Bunyaviridae* genus involves the viruses with a negative sense RNA whose genome comprises three segments: small (S; 830 bp), medium (M; 4415 bp) and large (L; 6865 bp). On the basis of the phylogenetic analysis of the segment S of Schmallenberg virus it was

asserted that the virus is most similar to Shamoda virus (Hoffmann et al., 2012). The segmented genome of *Bunyaviridae* creates a potential of re-assortment, which can lead to rapid genetic change in the virus population, as it happens with the new subtypes of influenza A viruses (Tarlinton et al., 2012).

### **Resistance to physical and chemical action (Anonymous, 2012 a)**

Data extrapolated from the California serogroup of Orthobunyaviruses.

Temperature: The infectivity is lost (or significantly reduced) at 50–60°C for at least 30 minutes;

Chemicals/Disinfectants: The virus is susceptible to common disinfectants (1% sodium hypochloride, 2% glutaraldehyde, 70% ethanol, formaldehyde);

Survival: The virus does not survive outside its host or its vector for long periods of time.

According to the epidemiological investigations, reinforced by what has already been known about the genetically related Simbu serogroup viruses, Schmallenberg virus affects the ruminants. The serological studies indicate that the virus is not zoonotic. The virus is transmitted into animals by vectors and then it is carried vertically during prenatal development.

### **Hosts**

Confirmed by the PCR or the virus isolation: cattle, sheep, goats, bison (Garigliany et al., 2012 a; Bouwstra et al., 2013).

Confirmed by serology only: red deer, roe deer, alpacas, mouflons, buffalos (Linden et al., 2012; Azkur et al., 2013).

Humans: The epidemiological and virological studies of the human populations considered to be at risk of infection did not demonstrate any evidence of zoonotic potential (Ducomble et al., 2012).

### **Transmission**

Epidemiological investigations indicate that some insects are the vectors.

Vectors: Schmallenberg virus genome was detected in several *Culicoides* species (Veronesi et al., 2013). The research carried out in Belgium in 2012 by De Regge et al. (2014) shows that subgenus *Avaritia* contains competent SBV vector species like: *C. obsoletus*, *C. scoticus*, *C. dewulfi*, *C. chiopterus*. Nevertheless, further information is required to determine whether the mosquitoes play a role in spreading the viruses. Vertical transmission across the placenta is proven. Direct transmission from animal to animal is very unlikely. Further research is still needed to confirm those transmission routes and to determine the insect species responsible for carrying the virus (Anonymous, 2012 b).

### **Clinical diagnosis**

The manifestation of clinical signs is varied by the species. For example, bovine adults have shown a mild form of acute disease during the vector season whereas congenital malformations have affected more species of ruminants (cattle, sheep, goat and bison so far). Some dairy sheep and cow farms have also reported diar-

rhoea. The SBV virus causes in adult animals (cattle): fever ( $>40^{\circ}\text{C}$ ), reduced milk yield, impaired general condition, anorexia, diarrhoea (Hoffmann et al., 2012). The recovery happens within few days for the individuals and after 2–3 weeks at the herd scale. In pregnant animals SBV can cause abortions, stillbirths and severe birth defects.

### **Pathological data**

The observed malformations in animals and stillbirths (calves, lambs, kids) are: arthrogryposis (joint stiffness and tendon shortening)/hydranencephaly, ataxia, paralysed limbs, muscle atrophy, brachygnathia inferior (undershot jaw, parrot mouth), ankylosis, torticollis (overstretched neck), scoliosis (curvature of the spine), kyphosis (extensive curvature of the thoracic vertebrae causing a hunchback appearance) and blindness (Herder et al., 2012; De Regge et al., 2013; Larska et al., 2013 a). The exact rate of malformation is not known and varies depending on the stage of gestation at the time of infection.

Lesions in malformed newborns are: hydranencephaly, hypoplasia of the central nervous system, porencephaly, subcutaneous oedema (calves). The symptoms can be summarised as arthrogryposis and hydranencephaly syndrome (AG/HE) (Garigliany et al., 2012 a).

### **Laboratory diagnosis**

The samples should be transported cooled and frozen (De Regge et al., 2013).

From a live animal for the detection of acute infection: EDTA blood, serum (at least 2 ml, transported in cool temperature).

From stillborns and malformed calves, lambs and kids:

- suitable organs for the detection of SBV: cerebrum, spinal cord, external placental fluid and umbilical cord (Bilk et al., 2012);
- antibody detection: pericardial fluid, blood (Garigliany et al., 2012 a; Garigliany et al., 2012 b);
- histopathology: fixed central nervous system, including spinal cord (Bilk et al., 2012).

### **Procedures**

Identification of the agent:

- real-time RT-PCR (Bilk et al., 2012; Bouwstra et al., 2013), commercial PCR kits are available;
- cell culture isolation of the virus: insect cells (KC) (Wernike et al., 2013 a), baby hamster kidney cells (BHK) (Breard et al., 2013), African green monkey kidney cells (VERO) (Loeffen et al., 2012; Bouwstra et al., 2013);
- transmission electron microscopy (Everest and Cooley, 2012).

Serological test on serum samples:

- ELISA: commercial kit available (Schelp et al., 2012; Breard et al., 2013);
- Indirect Immunofluorescence (FLI, 2013);
- Virus Neutralization test (Bouwstra et al., 2012; Loeffen et al., 2012).

### Vectors

The vectors involved in the transmission of the disease are biting midges (*Culicoides* spp.), which are among the smallest blood-sucking flies, with body lengths that rarely exceed three millimeters (Mellor et al., 2000). These insects are very similar to the mosquito. They feed on the blood of mammals and birds.

Elbers et al. (2012) examined different species of *Culicoides* in order to determine which ones are the vectors of the SBV. The study was performed in autumn 2011 in the Netherlands. SBV RNA was found in *C. scoticus*, *C. obsoletus* s.s and *C. chiopterus*. The researchers do not exclude participation of other *Culicoides* as the vectors of SBV. However, the experts believe that the high proportion of SBV-positive *Culicoides* spp. may help explain the rapid spread of SBV in Europe in 2011. The data from the Netherlands and Belgium have shown that the virus spreads rapidly reaching 80–90% seroprevalence in the exposed animals (Elbers et al., 2012). In Belgium, the speed of spreading the infection in the newborns was 28% (Gariglanly et al., 2012). The presence of the genetic material of SBV was confirmed in Belgium and Denmark (De Regge et al., 2012). The authors recognized *C. obsoletus complex*, *C. dewulfi* and *C. dipterus* as important vectors of the virus. Those results coincide with the outcome obtained by Rasmussen et al. (2012) who showed that SBV replicates in biting midges from *C. obsoletus* group (*C. obsoletus*, *C. dewulfi*, *C. scoticus*) and that those insects are the vectors of the virus.

In Poland, *Culicoides* spp. was also tested for the presence of SBV RNA (Larska et al., 2013 b). The authors have shown that the transovarial transmission of SBV in the midge population is possible which may explain the overwintering of the virus in insects in the absence of acute infections detectable in ruminants. Larska et al. (2013 b) also reported that the SBV spread into the colder and harsher climates of Europe may be related to the involvement of other vectors, such as that *C. punctatus*, detected in Poland.

### The potential impact on human health

In Robert Koch-Institute in Germany (Ducomble et al., 2012) the shepherds living in the epidemic area were tested for the presence of SBV antibodies. Sixty participants of the survey were interviewed for their patterns of contact with SBV-infected livestock and a blood sample was collected from each person. The RKI developed an immunofluorescence test (IFAT) and a virus neutralisation test (VNT). All analysed samples came out negative for SBV antibodies. In the National Institute of Public Health and Environment in the Netherlands (Anonymous, 2012 a) a similar study was performed. 301 people were tested for SBV antibodies by a VNT (234 of personnel working or living on SBV-infected farms, and 67 veterinarians). 229 people from the examined group of 301 had direct exposure to newborn calves, lambs and/or birthing materials from SBV-infected herds. 150 people reported exposure to biting insects. All carried tests came out negative for SBV antibodies, whereas high levels of antibody were found in serum from an infected animal that was used as a control.

ECDC rated the state of emergency in the EU (Anonymous, 2011 a; Anonymous, 2011 b; Anonymous, 2012 a) and fully supported the results obtained by RKI and



RIVM, which both concluded that the risk of infection of individuals exposed to SBV is absent or extremely low and that it is very unlikely that SBV puts humans at risk.

These assessment results are based on the following observations:

- the genetically most closely related viruses (Shamoda, Aino, Akabane) have never been associated with disease in humans;
- people who have been in close contact with infected animals have not reported any unusual disease;
- there is no evidence of any sero-conversion in people who presumably have been exposed to the virus.

### **The first reports in Poland**

The presence of SBV antibodies in Poland was detected in July 2012 for the first time. Kaba et al. (2013) collected blood samples from 230 adult goats from three western provinces of Poland bordering with Germany (West Pomerania, Lubuskie and Lower Silesia) and the ELISA test identified 21 seropositive goats.



Figures A, B, C – Malformations in intrauterine SBV infection in stillborn calf: scoliosis, torticollis, arthrogryposis and paralyzed limbs

Source: District Veterinary Inspectorate in Kamień Pomorski

In August 2012 two outbreaks of Schmallenberg virus infection were detected in Poland. They coincided with the introduction of two bulls imported from France into two herds located in West Pomerania and Silesia provinces. During routine tests in one of the bulls the SBV real-time RT-PCR positive result was obtained. In later studies of the second bull and both aforementioned herds the transmission of SBV was confirmed using real-time RT-PCR (Larska et al., 2013 a).

The first confirmed intrauterine fetal infection was detected in November 2012 in a stillborn calf from the West Pomerania province (Figures A, B, C). Another intrauterine infection with virus was confirmed by Larska et al. (2013 c). They examined one calf and twenty-nine stillborn lambs, dead or euthanised within 24 hours after birth. Samples for the study came from five farms located in West Pomerania and Silesia province, where SBV infection was confirmed using serological tests of mothers' plasma or testing cumulative milk from each herd. The authors made the conclusion that the Schmallenberg virus infection is widespread in Poland but accurate assessment of epizootic is complicated by the fact that the suspected infection of the SBV does not obligate to report it to the competent veterinary service. Therefore, it is impossible to assess the impact of SBV infection on ruminant breeding in Poland.

Another case of SBV infection was documented in May 2013 in West Pomerania. A stillborn calf was born. The fetus was deformed, the nature and extent of deformation indicated an intrauterine fetus infection as well as an infection of the mother with the Schmallenberg virus. The mother's plasma was tested and ELISA test was made to detect antibodies to SBV. The result of the test was positive.

### **Research on SBV**

Wernike et al. (2013) from Friedrich Loeffler Institute in Germany conducted a study which investigated the possibility of the oral infection as well as the immunity in convalescent animals and the cellular immune status after natural infection on the cattle. The researchers found that the immunity to re-infection with SBV persists for at least two months. What is more, the viral RNA is detectable for less than a week after infection and the oral instillation of SBV does not lead to infection. Besides, the viral RNA was detected in faecal, oral and nasal swabs taken from subcutaneously infected animals. They also discovered that SBV does not replicate in bovine PBL but it influences the lymphocyte homeostasis in the blood.

Hoffmann et al. (2013) examined whether SBV can pass into the semen of bulls. The excretion of SBV in semen may contribute to the spread of the virus by means of the natural and artificial breeding. The authors wanted to determine suitable methods for SBV detection in semen and check if the virus can really get into the semen. The results of their research suggest that it may be true. This situation could have considerable consequences on the trade of the semen from the bulls from SBV-affected regions.

De Regge et al. (2013) conducted studies of 90 malformed lambs and 81 malformed calves in order to compare the results of RT-PCR for the presence of SBV RNA taken from different organs and tissues. They showed that among the different organs of aborted lambs and calves, brain stem material is the most suitable matrix

for SBV detection by rRT-PCR. However, in other tissues we can also detect SBV RNA but with a variable degree.

At the present time the threat of the SBV should be fairly assessed so as not to cause unnecessary panic among the society. The symptoms observed in cattle are rather gentle and disappear quickly. Besides, the infection can also occur subclinically. On the other hand, explicit and rather spectacular symptoms occur when intrauterine infection of the fetus happens and those can arouse anxiety and emotions in people. It was determined that the virus does not threaten human health. However, the researches carried out to date show that it is worth preparing the society and the farmers to face the possible emergence of Schmallenberg virus infection in Poland.

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