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Emergence and consequences of Schmallenberg virus infection in Ruminants in North Rhine-Westphalia

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Budapest; 2013

Contents

1. Introduction

This thesis encompasses a literature review about the Schmallenberg virus and the reproductive consequences of infected ruminants in North-Rhine-Westphalia (NRW), Germany. The geographical origin of the Schmallenberg virus, clinical symptoms of the infection, transmission routes, detection methods and finally the global emerging are overviewed.

The Schmallenberg virus (SBV), firstly detected in August 2011 in a cattle herd close to Schmallenberg (NRW; Germany), is a newly emerged Orthobunya virus (family Bunyaviridae) that is transmitted by blood-sucking insects (mainly midges) and causes mild clinical symptoms in adult cows. SBV is closely related to the Simbu sero-group viruses, which includes the Akabane virus (VARELA et al; 2013) and is associated with unspecific clinical signs. Affected animals have fever, decreased milk production, loss of appetite, loss of body condition and diarrhea. In addition, spontaneous outbreaks of serious congenital malformations in aborted and stillborn lambs, calves and goat kids are reported. Most frequent macroscopic lesions of infected newborns are athrogryphosis, brachygnathia inferior, torticollis, kyphosis, lordosis, scoliosis and muscle hypoplasia. Predominately central-nervous-signs dominate, including cerebellar and cerebral hypoplasia, hydrancephaly, porencephaly, hydrocephalus and micromyelia (BILK et al; 2012). Malformations affecting lambs and calves exposed to the virus in pregnancy may lead to birth difficulties. Since the first identification of the new virus in 2011, SBV rapidly spread over large parts of North-Western Europe causing diseases and thus production losses.

The diagnosis of the Schmallenberg virus can be carried out by excluding other viruses causing similar diseases, the recognition of clinical signs and the use of a commercial ELISA kit for the detection of anti-Schmallenberg virus antibodies in the serum or plasma. The virus can infect cattle, sheep and goats, as well as bison and alpacas; the transmission is carried out mainly via blood-sucking insects such as midges.

Experiments exclude the direct transmission as a potential infection source for animals and humans. However, transplancental infection occurs frequently and leads to severe congenital malformations as described above.

A ruminant affected by the virus is not curable. Researchers have developed vaccinations but these are in general still in testing periods. However, the United Kingdom and France introduced vaccinations to prevent infections.

2. Scientific aspects of the Schmallenberg virus

2.1 Morphology and characteristics

Schmallenberg virus is classified in the Bunyaviridea family and the Orthobunyavirus genus (GOLLER et al.; 2012). Orthobunyavirus genus includes more than 170 viruses divided into 18 main sero-groups. Schmallenberg virus belongs to the Simbu sero-group (DOCEUL et al.; 2013). Bunyaviridae are approximately 80-120 nm in size with a spherical shape and the membrane of the viron is enveloped. The genome consists of three segments of negative-sense single-stranded RNA containing the L (large, 6870 nucleotides), M (medium, 4310 nucleotides) and S (small, 860 nucleotides) segment (Fig. 1). The L segment encodes the RNA-dependent RNA-polymerase L. The M segment of the viron encodes a precursor polyprotein that is cleaved into the envelope glycoproteins G1 and G2 as well as the NSm, a non-structural protein. The third segment, S encodes the nucleoprotein N and the non-structural protein NSs (ELLIOTT et al.; 2013, SCHMALJOHN et al.; 2007). Virus particles are generated of four structural proteins responsible for the transcription and the replication in the cytoplasm. Two surface glycoproteins Gn and Gc and the viral polymerase complex are composed of the polymerase L protein and the nucleoprotein N. The viral genome is present as a ribonucleoprotein (RNP) inside this complex.

The virus is heat labile and is inactivated at 56 ° C in a few minutes. At 37 ° C, it loses about 0.3 logs of its infectivity per hour. The virus is not acid resistance and may be inactivated by conventional alcohol-based disinfectants and detergents (MELLOR et al.; 2008). For survival of the virus a vector or a host is necessary.

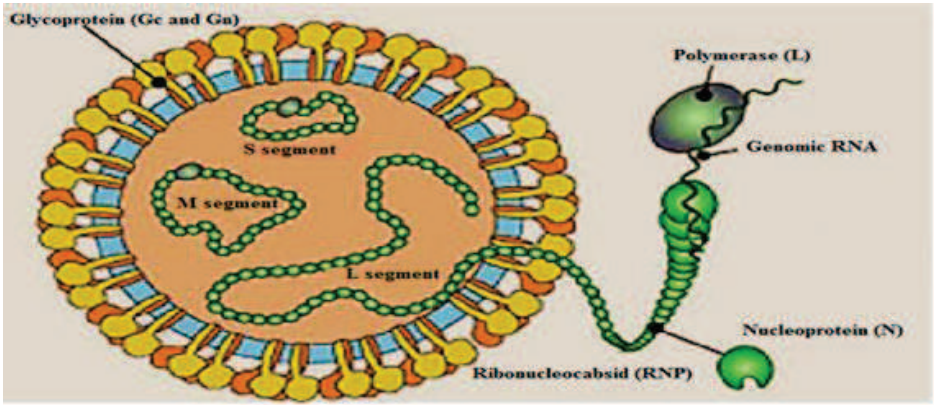


Figure 1. Model of a Bunyavirus (ViralZone; 2012)

2.2 Pathogenicity

Since the Schmallenberg virus is a newly discovered virus it doesn't have a scientifically defined set of features yet. However, it's pathogenicity and life cycle is supposed to be the same as in other related Bunyaviruses. The prototypic Bunyavirus is called the Bunyamwera virus (BUNV). BUNVs are used as models for investigation of the molecular and cellular biology of pathogens within the Bunyaviridae family (BRIDGEN et al.; 2001).

This group with negative-stranded RNA viruses has the unique feature, that is the on-going protein synthesis for the transcription of functional mRNAs (WALTER et al.; 2011). Characteristics of BUNV also refer to the pathogenicity and the life cycle of the Schmallenberg virus. BUNVs can replicate in both vertebrate and invertebrate cells in tissue culture. In mosquito cells, there is no cytopathology and persistent infection is established, whereas in mammalian cells infection is lytic and leads to apoptosis. Therefore, the virus is able to form lytic plaques in cells of vertebrate origin but not in those derived from insects (SZEMIEL et al.; 2012). Bunyaviruses exclusively replicate in the cytoplasm. After recognition at the cellular receptors by the surface glycoprotein Gn and Gc, virions enter the cell by endocytosis (ELLIOT; 1990). Change in pH inside the cellular vesicles induces a conformational modification of the viral glycoproteins and Gc fusion peptide exposure (PLASSMEYER et al., 2007). After the fusion of the viral envelope with the membranes of the endosomes, the ribonucleinprotein (RPN) reaches into the cytoplasm and the primary transcription for producing viral mRNA starts (BOULOY; 1990). Translation of the viral mRNA by the host cell ribosomes leads to a production of viral protein L and N. These proteins are responsible for the replication of the viral genom. Glycoproteins Gn and Gc form complexes in the endoplasmic reticulum. After transporting the glycoproteins to the Golgi-apperatus, glycosylation will be completed. From free nucleotides L polymerase is able to produce complementary copies of the whole viral genom for producing higher quantities of viral genoms. Maturation of the viral particles occurs via budding into the membrane of the Golgi- apparatus (WALTER et al; 2011). For the Orthobunya viruses, the non-structural protein (NS) seems to be the major virulence factor. NSs inhibit protein-synthesis and host cell's antiviral response due to the intervening RNA polymerase. During transcription, Interferon-type 1 proteins are produced causing apoptosis (BRIDGEN et al; 2001).

The virus is only detectable for a short time in the blood of the infected adults. Once the animal is infected by SBV, the immune system protects the organism from further re-infection. Despite

the recently available wide knowledge, further investigations are needed to estimate the duration of the immune protection.

2.3 Transmission

Epidemiological studies indicate ceratopogonid midges being responsible for the major transmission route of the Schmallenberg virus. This is also typical for the related Shamonda, Sathuperi, Aino, and Akabane viruses (REUSKEN et al.; 2012). Primarily culicoides subspecies such as C. obsoletus complex, C. chiopterus and C. dewulfi are involved in the transmission (ELBERS et al.; 2013). The peak population of the adult midge stage occurs during late summer and autumn; therefore, acute symptoms caused by viruses transmitted by culicoides are prevalent during that period (ELBERS et al.; 2013; DE REGGE et al.; 2012). The initial replication of the virus inside the vector occurs in the midgut epithelium. Subsequently, the virus disseminates into the hemocoel and reaches the hemolymph. Finally, the virus enters cells of the salivary glands, replicates and reaches the salivary ducts where it can be transmitted to the final host. Different factors, such as vector/host dynamics, climate and immune status of the ruminants population, play a significant role in life cycle of this arbovirus. At the end of 2012 researchers of the Friedrich-Loeffler-Institut detected genetic material of the virus by using RT-PCR for analyzing the sperm of bulls (BEER et al.; 2012).

An important role in transmission of the virus is the infection during the vulnerable stage of pregnancy. This infection occurs in sheep probably during the 4^{th} to 8^{th}, while in cattle during the 8^{th} and 14^{th} week of pregnancy and causes severe malformation of the fetus, or even stillbirth. Experimental oro-nasal inoculation of two calves did not cause an infection and therefore excluded the possibility of a direct transmission of the virus. To determine the incubation time of SBV researcher infected cattle and sheep in the course of an experiment. These animals showed no clinical signs at all or only mild symptoms at three to five days post inoculation. The result of this experiment was an incubation period of between one and four days and viraemia lasting for one to five days. (DOCEUL et al; 2013)

According to the epidemiological investigations, reinfection can be excluded due to the knowledge of genetically related Simbu sero-group viruses.

Serological studies of the EU Centre for Disease Prevention and Control indicate that the Schmallenberg virus is not zoonotic and therefore no risk assumed for human beings. In

addition, the Robert-Koch Institute reported no evidence of human infection by target groups such as sheep farmers (DUCOMBLE et al.; 2012).

2.4 Detection methods

During the summer of 2011, the Federal Research Institute for Animal Health laboratory for bluetongue diseases at the Friedrich-Loeffler-Institut of Diagnostic Virology on the Isle of Riems notified, based on clinical signs within a dairy cowherd in North Rhine-Westphalia a new introduction of bluetongue disease. The herds showed clinical signs but after several days, these disappeared. A Schmallenberg virus induced pathology cannot be differentiated from infections with Akabane virus (AKV) (HOEPER et al.; 2012). Only its molecular identification by using PCR can help to give an etiological diagnosis (HOFFMANN et al.; 2012).
Detection of viral nucleotides from diseased animals, placental fluid, umbilical cord, cerebrum, and spinal cord were examined. The virus detection from the spleen, cartilage, placental fluid, stomach and meconium were not suitable because they rarely revealed positive PCR results (BILK et al.; 2012). Beside the accurate PCR-method, commercially available indirect enzyme linked immunosorbant assays (ELISA) may be used for testing serum samples (BREARD et al.; 2013). For detection of an acute infection in living animals, the laboratories use EDTA blood and serum while in case of stillbirth and malformed calves, lambs and kids, virus detection in samples from the cerebrum, brainstem and amniotic fluid are useful. From live newborn amniotic fluid, placenta, sometimes meconium samples can be used to detect the virus. In dairy herds, also individual milk samples and bulk milk samples can be useful to prove antibodies of SBV (HUMPHERIES et al.; 2012).
Further possibilities to detect seropositivity are indirect immunofluorescence (IF) and virus neutralization (VN) tests. The VN test displays a specificity of >99% and a sensitivity of approximately 100 %, and is useful to screen for antibody levels (LOEFFEN et al.; 2012).
For immunohistological detection of the Schmallenberg virus protein in neurons an antibody detection of Tinaroo virus, which is a subspecies of the AKV and belongs to the genus Bunyavirus, was helpful to confirm a positive PCR-result (PEPERKAMP et al.; 2012). Furthermore, in vivo- models to study the pathogenesis of SBV by cell culture isolation of the virus with insect cells (KC), baby hamster cells (BHK), monkey kidney cells (VERO) can be used to test the infection (WERNICKE et al.; 2012).

It is not possible to use the VN test and plaque reduction neutralization test for the diagnostics of large number of samples because these methods are too time-consuming (4 to 6 days) and cannot be automated (DOCEUL et al.; 2013). It is therefore recommended to use the ELISA-technique for fat and reliable results. This method can help to determine monitoring and surveillance in ruminant herds especially if animals are imported or exported from the country (BREAD et al.; 2013).

3. Emergence of the Schmallenberg virus

The European Food Safety Authority (EFSA) and Friedrich-Loeffler-Institute authored a technical report with the collection of all epidemiological data of the Schmallenberg virus infection within the European Union (EFSA; 2013). This analysis provides an overview of the SBV situation between the 1st of August 2011 and the 30th of April 2013 and also informs about newly affected cattle herds thereafter. It allows a survey about the current distribution, the frequency of occurrence and due to this, the verisimilitude processing of the virus. A cautious interpretation of these data is necessary since the numbers of reports of a virus occurrence depend on the fact whether the disease has to be officially notified or not. In some countries, there is still no obligation to inform about infections. The Federal Council of Germany has introduced the official reporting requirement in March 2012.

Three ranking models are used to grade SBV in regards of the animal welfare and animal production such as milk yield and rates of dystocia: the geographical, the seasonal and the projection model.

1. The geographical model observed dynamic spreading in Europe during 2011in terms of duration of the transmission period and the number of infected holdings within a region.

The use of the geographical model can be difficult as there is a lack of knowledge of the virus itself and thus the correct identification of it within affected holdings.

2. The seasonal model operates with environmental factors such as external temperatures, vector population and keeping conditions.

For the geographical and seasonal model within holding transmission, bluetongue virus (BTV) parameters are suggested for the virus transmission and spread. That means that the optimal temperature for transmission will be between 18°C and 19°C.This climate is in almost all European countries present and increased vector borne transmission is supposed. Further factors such as the reinfection due to the overwintering process of biting midges also needs to be taken into consideration.

3. The projection model is based on the geographical spread mode and reported affected holdings. The analysis of impact is limited to regions where calving and lambing data were available to EFSA. The model predicts that the regions that have the highest infection and impact figures in cattle and sheep as well as reported “arthrogryposis hydrancephaly syndrome” (AHS) cases will be found generally in regions with large numbers of holdings (high livestock density). Therefore the SBV infection expanded mostly during winter 2012 and spring 2013 in most European countries (ROBERTS; 2012).

North Rhine Westphalia (NRW; Germany) the country of first detection, reported 572 cases. Of these reported infections, 286 belonged to cattle -, 273 to sheep- and 13 to goatherds (REINKING et al; 2013). In comparison with the total number of ruminants in NRW this number is not too high. In January 2013 to August 2013, FLI reported eleven cattle and two sheep infections. In regions with SBV confirmed holdings, assuming a high prevalence of infection and therefore post infection immunity, the impact of new infections in the calving and lambing season of 2012-2013 should be lower. Compared to this in unaffected regions or regions with low prevalence with suitable temperatures for herd transmission by vectors and a high density of susceptible species (cattle and sheep) SBV infection is likely to spread (Fig.2).

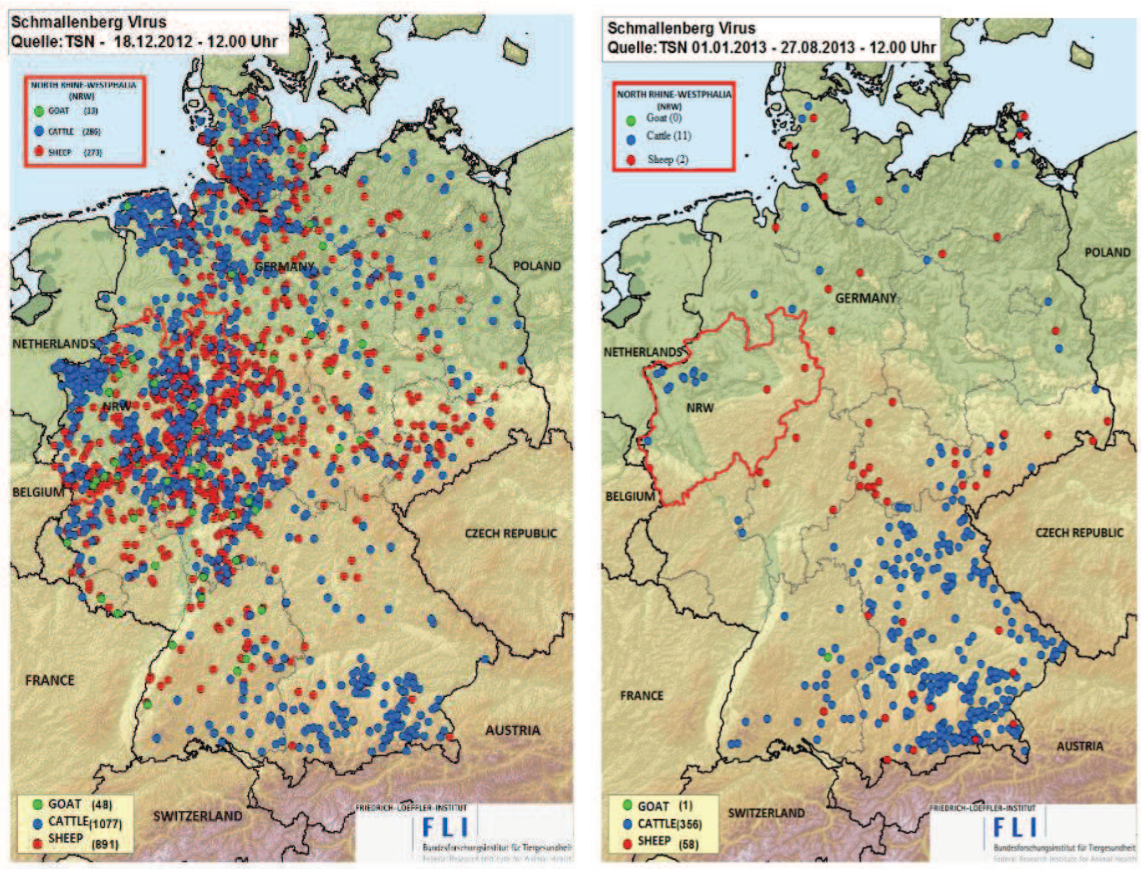


Figure 2. Location of farms with SBV-positive ruminants in Germany as diagnosed by PCR-technique (left: 2011-2012; right: 2013) (Figure from FLI with modification)

3.1. Origin

The history and geographical origins of the Schmallenberg virus raise numerous questions. The causes of SBV emergence in northern Europe remain unknown.

Phylogenetically the closest relatives of the Schmallenberg virus are Sathuperi, Douglas, Shamonda, Akabane, Aino, Peaton or Sango viruses (REUSKEN et al.; 2012). However, none of these viruses were normally identified in Europe. Akabane and Aino viruses are distributed in the Far East and Australia, but Akabane virus has been found recently in Israel and Turkey. Shamonda virus was detected in Nigeria in the 1960s and has been isolated only in Japan in 2005 (YANASE et al., 2005). It is surprising that these viruses emerge into European regions so far away from their original areas.

Although SBV has only been discovered recently, there is no doubt that its origin is more ancient and that it might have co-evolved with other closely related viruses. Until now, viruses belonging to the Simbu sero-group have not been completely studied and their epidemiological features are still poor. Nevertheless, phylogenetic analyses based on samples taken in different regions of the world at different periods suggest that these viruses evolve slowly.

Shamonda virus is thought to be a reassortant containing the S and L genomic segments from SBV and the M segment from an unclassified virus (GOLLER et al.; 2012). Such a reassortment phenomenon has been described previously between viruses of the genus of Orthobunyaviridea (YANASE et al.; 2010).

Peaton virus (belonging to the Simbu sero-group) seems to be derived from an ancestor generated by a reassortment between Akabane and Aino viruses (YANASE et al.; 2003).

The emergence of SBV is a reminder that bunyaviruses and particularly orthobunyaviruses are a potential threat for European livestock. Akabane and Aino viruses are already present in the Mediterranean region and might be introduced into the rest of Europe. Consequently, the surveillance of livestock and vector populations is critical to monitor the emergence of such viruses in Europe.

The fact that Schmallenberg virus has emerged in the same region of Europe as the Bluetongue virus (BTV)-8 in 2006 and later BTV-6 and−11, and may suggest that the viruses followed the same routes of introduction (DOECEUL et al.; 2012). It is possible that SBV was present in a region of the world where no and/or rare clinical signs were manifested or reported in the native population before the outbreak was firstly detected in Schmallenberg. In consequence, there is no doubt that Europe will face more and more frequently with this type of emergence.

3.2 Economic aspects

The export of German breeding cattle in 2012 dropped by over 36 percent in comparison to the previous year. In 2012, only 66,320 breeding cattle were exported, while there were 104,810 animals during the preceding year (Federal Statistical Office, Wiesbaden, Germany, 2013). (Fig. 3)

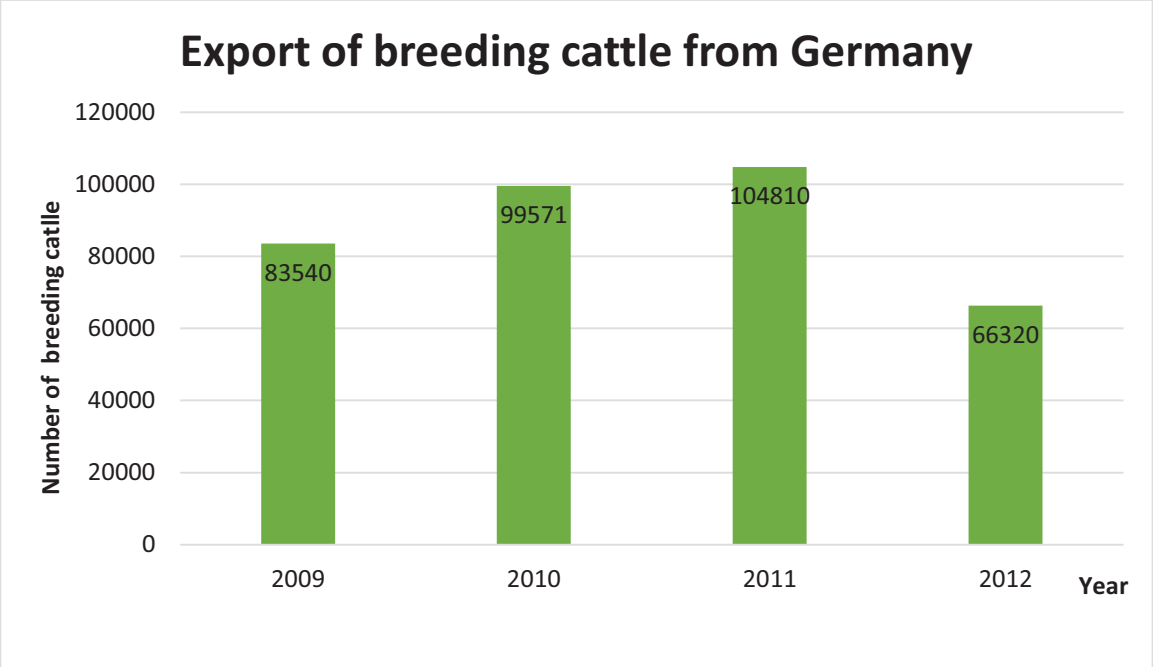


Figure 3. Export of breeding cattle from Germany (Federal Statistical Office, Wiesbaden, Germany, 2013)

This indicates the immense consequences of the virus for the farmers and the livestock markets. The largest falls were reported from Morocco (- 60%) and Italy (-52%). Of the five major exporting countries for German breeding cattle, the virus did not infect the export business of two countries: The Netherlands even increased its imports by 2% and Turkey ordered almost 950% more animals from Germany.

Schmallenberg virus also negatively affects other European breeding cattle export nations such as France, the Netherlands and Austria. The export of breeding cattle from the EU decreased by around 40% during 2012 (GOOS; 2012).

Maintaining an efficient surveillance would be essential to further describe the progression of the epidemic and its impact on the breeding industry.

The export of semen from countries where SBV is present might be a proper indicator that represents the risk of contamination. Therefore, it is essential to develop a sensitive test for the detection of SBV RNA in semen and to perform further studies to determine the risk of transmission of SBV via this route and the impact of the virus on fertility. As shown in Fig. 4,

bovine semen export decreased with 9.8 % in comparison to the previous year (Federal Statistical Office, Wiesbaden, Germany, 2013).

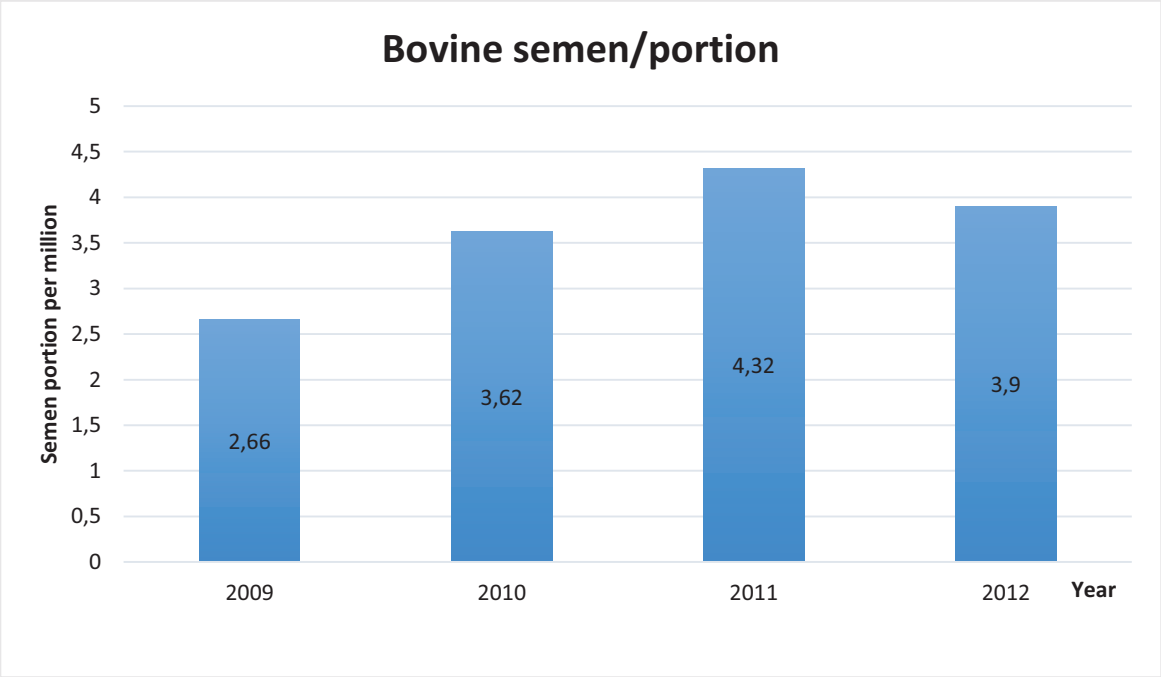


Figure 4. Bovine semen export from Germany (Federal Statistical Office, Wiesbaden, Germany, 2013)

4. Clinical signs and necropsy findings

The clinical signs of affected animals markedly varies in severity and between species and age. Some animals can be without any clinical signs, therefore, the existence of clinical signs alone is not a reliable indicator for the detection of a Schmallenberg virus infection. However, clinical signs help to consolidate possible epidemic consequences.

4.1 Clinical findings and gross pathology in cattle, sheep and goats

In adult animals, the infection with Schmallenberg virus and the subsequent outcome of this infection mostly occur during the vector active seasons. Infected animals can inconspicuous or may show one or more of the following clinical signs:

- anorexia
- hyperthermia (> 40°C)
- impaired general condition
- reduced milk yield (up to 50%)
- diarrhea
- increased incidence of abortions

These symptoms usually disappear within a few days. Affected herds can have a transient viraemia and may recover within two to three weeks. Individuals had even recovered after several days.

Infected sheep and goats seem to be very mildly affected, although there is anecdotal evidence of milk drop in milking sheep (DOMINGUEZ et al.; 2012).

Acute disease may be difficult to recognize especially when the only symptom is a milk drop in dairy herds. Instead, the presence of infection may not even be realized until congenital deformities due to vertical transmission are seen in aborted or full term calves and lambs.

Cattle infected by SBV in their adolescence are more conspicuous to show clinical signs than goat or sheep. Cattle with acute infection show mild symptoms such as fever and a drop in milk yield. These symptoms mainly have been observed during the vector-active season for only a short period (JUST et al.; 2013).

Compared to that the acute infection in goats and sheep is processing inapperant and unobtrusively. This subclinical process makes it difficult to diagnose SBV early enough to prevent malformed offsprings.

4.1.1 Significance according to the reproductive state

Dystocia due to malformed fetuses can cause severe consequences according to the consecutive reproductive performance in SBV infected herds.

A transplancental infection in the tetragonic determination phase causes malformation of the embryo/ fetus.

According to the strong relationship of SBV to the Akabane-virus this period can be determined in sheep during the day 28 to 36 (50), in goat between day 30 and 50 and in cattle between day 75 to 110 (150) (DOCEUL et al.; 2013).

An infection outside this period probably does not or only seldomly causes consequences.

The malformations associated with SBV infection is the "arthrogryposis hydranencephaly syndrome" (AHS).

Athrogryposis is a multiple congenital contracture characterized by bent limbs and joint.

Contractures are present at birth and due to the fixing joints in abnormal positions and restricting of their movements it can lead to severe parturition problems.

Torticollis, scoliosis, brachygnathism, and kyphosis may coexist with arthrogryposis.

In these cases, an assisted lambing or calving under strict hygiene management is advisable.

Hydranencephaly is defined as a condition in which the cerebral hemispheres of the brain are absent and replaced by sacs filled with cerebrospinal fluid. Usually, cerebellum and brainstem are formed normally (VAN DEN BROM et al.; 2012).

Most live neonates have central nervous system degeneration and muscle lesions that prevent the SBV infected animal from standing or suckling.

Lesions in the central nervous system are manifested clinically as blindness, nystagmus, deafness, dullness, slow suckling, paralysis, and incoordination. Mildly affected calves or lambs may improve their mobility with the time. However, most of the infected animals die within 6 months as a result of blindness and other neurological defects (HARTLEY et al.; 1977). In case of dead fetuses, foetotomy is advised.

The neurological disorders reported as severe central nervous system lesions, severe dysfunctions of the cerebral cortex, basal ganglia and mesencephalon, severe porencephaly or hydrancephalus generally lead to death of the animal within several hours or maximal several days after birth. In case of twin gestations in sheep, one twin can suffer from arthrogryposis hile the other from neurological disorders. One twin can also be born malformed while the other will be able to live being only affected by a delayed growth (DOCEUL et al.; 2012).

Malformations caused by the Schmallenberg virus are expected to be present only in offspring of infected non-immune dams. An infected mother develops antibodies, which will save the next offspring, as an adverse effect on the fetus will prevent possible re-infection.
However, it is not known how long the natural immunity of an infected animals will remain.

4.2 Clinical findings and gross pathology in newborn calves, lambs and kids

Clinical signs in newborns and fetus appear in form of arthrogryposis, hydranencephaly, brachygnathia inferior, ankylosis, torticollis, scoliosis and subcutaneous oedema. Some fetuses are born with a normal outer appearance but have nervous signs such as a 'dummy' presentation or blindness, ataxia, recumbency, and inability to suck and sometimes convulsions (DOCEUL et al.; 2012). These abnormalities in live-born offsprings can result in immediate or long-term welfare problems.Additional care during the rearing period in less severely affected animals may be required. In severe cases, euthanasia must be taken into consideration.
The severity of fetal malformation depends on the time of SBV infection during gestation. The maximal damage occurs, when neural tissues are differentiating approximately during the first third of pregnancy (STEUKERS et al; 2012).No visible clinical signs are present in the neonates if infection occurs while the immunestate of the fetus is able to down regulate the infection (GARLIGLIANY et al; 2012).
There are different potential diseases causing similar clinical signs as the Schmallenberg virus. It is therefore not accurate to diagnose a SBV infection purely by recording the clinical signs in newborns.

Picture 1. Newborn calf with SBV infection (Wilson et al.; 2008)

4.2.1. Major malformations in the central nervous system and the locomotion system

The most predominant central nervous system lesions caused by a Schmallenberg virus infection are malformations such as hydrocephalus internus, hydranencephaly, porencephaly, cerebellar hypoplasia, micromyelia and multicystic encephalopathy (HAHN et al.; 2013).

Hydrochephalus internus is defined as an 'abnormal accumulation of CSF in the ventricular system of the cranial cavity' (MAXIE et al.; 2007). A so-called communicating hydrochephalus results due to an increased amount of fluid and improper absorbance ability. Non-communicating hydrocephalus occurs when CSF flow is obstructed within the ventricular system or in its outlets to the arachnoid space, resulting in impairment of the CSF from the ventricular to the subarachnoid space (ZACHARY; 2007). In this case the hydrochephalus can be defined based on the on the mechanisms as 'an increase in volume of CSF' due to compensation or obstruction (SUMMERS et al.; 1995). Due to the compensatory mechanism of this circumstances, a loss of brain tissue as a result of tissue destruction or a developmental impairment is reported.

Hydranchephaly differs from a hydrochephalus internus because of a 'complete or almost complete absence of cerebral hemispheres, leaving only membraneous sacs filled with CSF and enclosed by meninges' (MAXIE et al.; 2007). It is impossible to distinguish between the grey and white matter according to the severe damage of the cerebral cortex. In some cases the neural tissue is almost abscent and the cerebral sac only consits of astroglia, pia mater and blood vessels (SUMMERS et al.; 1995). This malformation displays a 'full-thickness necrosis of the hemispheres' (MAXIE et al.; 2007). The remaining neopallium, which is a thin, nearly transparent membrane that collapses on the underlying brain tissue when the brain is removed, represents another definition of hydranencephaly. Despite severe lesions in the cortex, hippocampus and brain stem may remain intact (SUMMERS et al.; 1995). For hydranencephaly the term'fluid-filled bubble-like hemispheres' is also used (HERDER et al.; 2013).

Porencephaly is also a reported malformation caused by a Schmallenberg virus infection. This term is defined as a 'hemispheric defect originating during fetal life and antedating the acquisition of a mature astroglial response or completion of convolutional development' (HERDER et al.; 2013).

Cerebellar hypoplasia represent a non-physiological small cerebrellum caused by abiotrophy, hypoplasia or atrophy (SUMMERS et al.; 1995, ZACHARY; 2007).

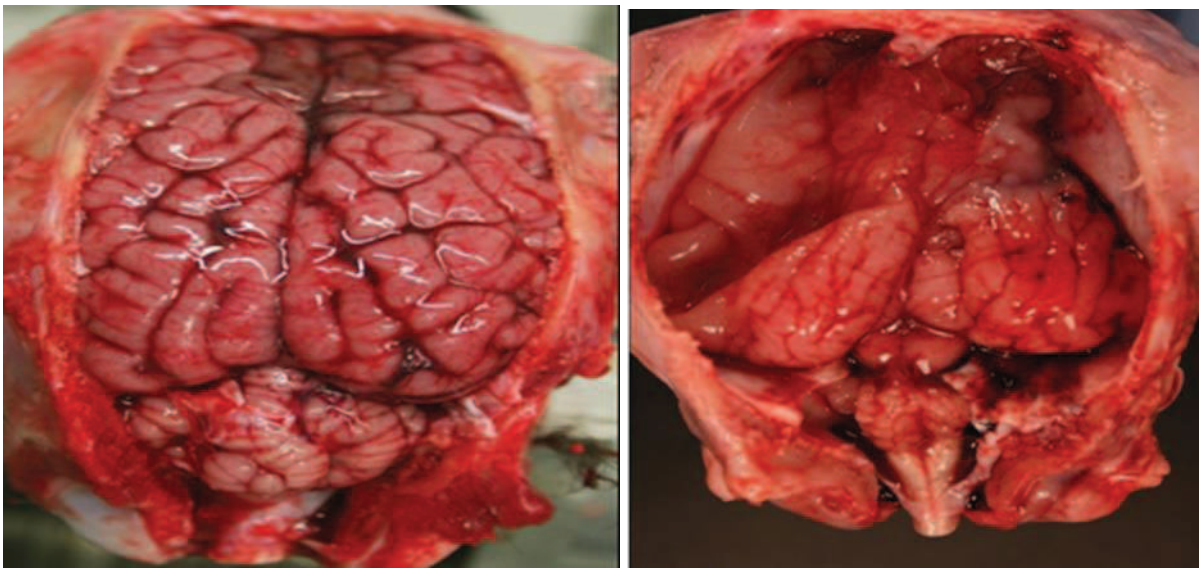
The normal cerebellum weight ranged between 10 and 12% of the total brain weight and smaller values indicate malformations, like cerebellar hypoplasia or abiotrophy (SCHATZBERG et al.; 2003).

Abiothrophy is characterized as a 'premature senescence of formed nervous tissue as disturbance of a normal development' (MAXIE et al.; 2007) and the primary affects the Purkinje neurons (WHITE et al.; 1975). SBV infected aborted and neonatal lambs are displaying a similar morphology with multicystic lesions in the CNS (HERDER et al.; 2013).

Cerebellar atrophy is characterized by a degeneration of Purkinje and Golgi cells while the size of the organ is macroscopically normal (MAXIE et al.; 2007).

Micromyelia is a size-reduced spinal cord in diameter. In infected animals a lack of large areas in the grey matter with a decreased amount of neurons appear. Occasionally, this process can distribute within the white matter.

All of these pathological changes are responsible for the severe neurological disorders. Most of the affected animals are unable to reach maturity because of inability of food intake and or incoordination.



Picture 2,3 Left: Open skull of a lamb Right: The cerebrum transofrmed into fluid filled sac.The Cerebrum is too small (Peters;2012)

The prominent malformations of the skeleton system of SBV infected neonates are arthrogryposis, brachygnathia inferior, torticollis, kyphosis, lordosis, scoliosis, and muscle hypoplasia (HAHN et al; 2013).

Arthrogryposis multiplex, or "curly calf syndrome" describes the permanent contracture of the joints of the front and/or hind limbs. This is often combined with an abnormal curvature of the spine as scoliosis, a lateral abnormal position of the spine. In severe cases of scoliosis, this

malformation can lead to diminishing lung capacity, putting pressure on the heart, and restricting physical activities. The torsion of the neck, torticollis is also observed in severely affected animals.

Kyphosis and lordiosis describes the pathological position of the Spine. Kyphosis is an outward and the lordiosis an inward curvature of the whole or partial spinal column. In addition, myofibrillar hypoplasia was reported in lambs and calves. The skeleton deformation leads to and rearing difficulties.

The predominant malformations are described as the "arthrogryposis hydrancephaly syndrome" (AHS) and is more typical for sheep than for cattle (METZNER; 2012). Classical signs of AHS include: stillbirth, premature birth, mummified fetuses, hydranencephaly, arthrogryposis, ataxia, paralysis, muscle atrophy, joint malformations, torticollis, kyphosis, scoliosis, lordosis behavioral abnormalities and blindness.

While in sheep flocks are affected and the infection often occurs in several animals, in cattle herds it often only affects single animals.

Picture 4, 5. Left: Lamb affected by arthogryphosis (persistent flexion of the joints) Right: Brachygnathia inferior (Peters and Köß ;2012)

4.3 Histological findings

Histological studies revealed astrogilosis and microgliosis in the central nervous system of SBV infected cattle and sheep. Also in both species, some cases of myofibrillar hypoplasia in the skeletal muscle were detectable. Exclusively in sheep, the studies indicated lymphohistological inflammation in the central nervous system and glial nodules in the hippocampus and mesencephalon (HERDER et al.; 2012). Meningoenchephalitis and poliomyelitis was

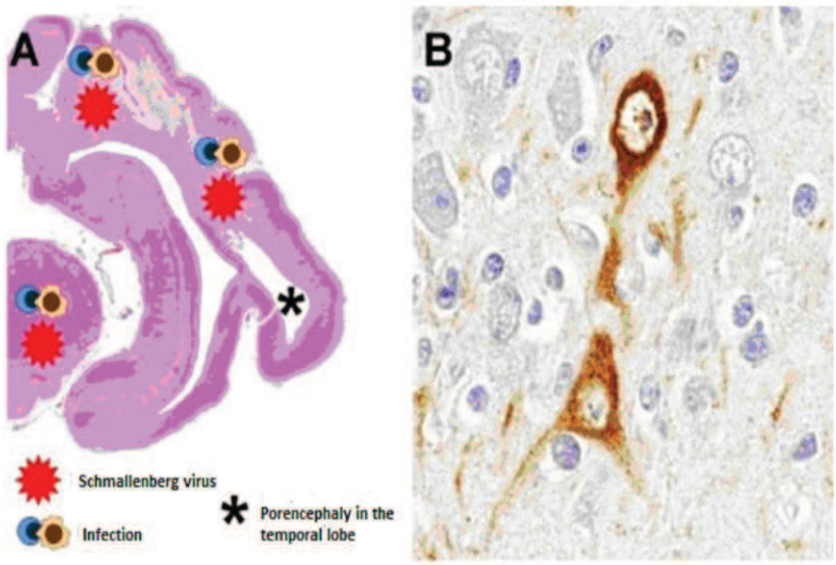
demonstrated by histological examination in a ten-day old SBV positive calf (PEPERCAMP et al.; 2012).

Immunochemistry and in situ hybridisation performed on brain sections identified neurons as the major target for the SBV replication (HAHN et al.; 2013).

Scientists from the Institute of Pathology, University of Veterinary Medicine Hannover, the State Veterinary Office Arnsberg and the College of Medical, Veterinary and Life Sciences at the University of Glasgow have analyzed samples from 82 newborn and naturally infected calves, lambs and goats on inflammation in the brain and spinal cord. Only in 15 animals they could prove inflammation in the central nervous system. In all 15 animals, they found a lymphohistiocytic meningoencephalomyelitis (HERDER et al.; 2012).

Particularly the midbrain (mesencephalon) and the parietal and temporal lobes were affected. This indicates that different brain regions are differently susceptible to infection with the Schmallenberg virus. Most often, CD3-positive T cells, CD79a-positive B cells and CD68-positive macrophages were found. The inflammation in the brain and spinal cord rarely occur in infected animals, this indicates that the time of infection and thus the development of the immune system of the fetus plays a crucial role in the disease process (HERDER et al.; 2013).

Typical brain malformations in the SBV infection are porencephaly (cystic cavities in the brain, Pict. 5) and hydranencephaly (bladder brain). Futhermore associations of brain malformations with destroyed myelin sheaths and axons were reported. In the deformities, the virus-infected nerve cells lose their structure.



Picture 6. Schmallenberg virus infection, which is transferred to the fetus during pregnancy can cause porencephaly (asterisk, A) in the brain of the fetus. In addition, inflammation and viral proteins in the three brain regions midbrain, temporal and parietal lobes (A) could be demonstrate. Immunohistochemical methods, the virus in the tissue section in the nerve cells (brown signal B) / (Herder; 2012)

5. Treatment and prevention

Treatment against Schmallenberg virus-infection is currently not available. During the vector active season an infection can cause a mild form of acute disease in cattle. The animals generally recover within a few days. Adult animals infected by SBV usually survive mild viraemia or the infection remain inapparent (BEER et al.; 2012). Animals that become infected during their pregnancy, are subject of malformed offsprings or stillbirth. The deformities or malformations are often life threatening and can cause severe distress to the newborns. In acceptance to the animal welfare, culling should be initiated to avoid unjustifiably pain to the animals.

Vaccination is a preventive measure that could reduce the impact of SBV. Until recently, no efficient or reliable prevention against SBV infection is available. Such vaccines are still in the developmental phase. Several vaccine manufacturers and research institutions are working on inactivated vaccines. Therefore, the harmlessness and effectiveness of the vaccines are tested also in pregnant animals before they can be approved and implemented. Nevertheless, the cost of vaccination for the livestock industry might not be justified since SBV seems to be a low impact disease. (ACKERMANN; 2012). Although vaccines have been developed against Akabane disease but management of their outbreaks relies mainly on the sentinel monitoring.

Susceptible animals can be protected against biting midges and mosquitoes to reduce the risk of infection. Direct application of synthetic pyrethroid pour on insecticides are advised as they could control the vector population properly. Such treatment is also helpful to reduce other culicoides- and thereby act in the prevention of their transmitted diseases, for instance the bluetongue virus disease (CARPENTER et al.; 2008). However, the control of midges with pour-on medications has been dramatically unsuccessful with no noticeable reduction in the density of biting midges (BAUER et al., 2009). It also might be recommended to keep the animals during the critical periods of pregnancy insight during twilight and night. This procedure can possibly reduce the exposure to SBV- infected midges (TARLITON et al.; 2012). Unfortunatley, control of midges is unlikely to be effective, given that they are very widespread, and appear to be very effective at spreading the virus.

A complete transmission control is therefore not possible. Delayed breeding may decrease the number of foetal malformations.

SBV has a limited impact on animal health, therefore trade restrictions have not been advised and are regarded as unjustified by the European Union (EU) and the World Organisation for Animal Health (OIE) (European Comission; 2012). SBV is transmitted by a vector that is widespread within Europe and movement bans would be ineffective.

However, many countries outside the EU have imposed restrictions on the import of living animals and products from the EU such as semen and embryos. Recently, SBV RNA has been detected in the semen of naturally infected bulls and SBV infection was reported in calves inoculated experimentally with SBV positive semen as detected by RT-qPCR (HOFFMANN et al; 2012). Therefore, semen export from countries where SBV is present might represent a risk of contamination. In order to diminish this risk, it is essential to develop a sensitive test for the detection of SBV RNA in semen. It is advisable to perform further studies to determine the risk of SBV transmission via this route and the impact of the virus on fertility.

These findings strongly differ from those caused by the related Akabane virus. Experimentally with Akabane virus infected bulls did not show semen contamination, suggesting that semen can not transmit Akabane virus infection.

Up to now the reported number of infected herds and the losses of animals, is still not a really extential thread for farmers, when compared with other epidemic diseases.

As long as there are no practical ways of vaccination, the only advice, which can be given to farmers, is to keep their pregnant animals in holdings especially in the time when midges are most aggressive (which is during twilight and nights) (TARLINTON; 2012). This is of special importance, especially when the herds are located in regions where possible cases of SBV infection have been reported.

The still unknown circumstances of the infection way of the Schmallenberg virus shows dramatically the thread that new infections may appear even in far laying regions.

Until recently, it is not known, how the midges around the city of Schmallenberg got infected by this virus. Possible ways that brought the SBV to NRW might have been the import of infected animals, semen or other organic material from regions known to be affected by analogous viruses. Therefore, at least as important as the development of a serum is the development of a test, which can indicate potential infected organic carriers. This test should be easy to handle and should not be too expensive.

6. Discussion

The aim of the present thesis was to summarize the main features of Schmallenberg virus infection and to focus on its effect on reproductive disorders and resulting malformations in ruminants in the region of North Rhine-Westphalia.

Schmallenberg virus is the first Simbu sero-group member of Orthobunyaviruses, which was detected in Germany (CONRATHS et al.; 2013). This feature makes the virus unique and gives room for interpretation of the virus emergence worldwide. Orthobunyaviruses of cattle are common in Australia, Asia and Africa, and usually causing very mild clinical symptoms in adult animals. If pregnant animals are infected, sometimes considerable congenital damages, premature births and fertility disorders may occur. Schmallenberg virus is not representing a zoonotic danger. It has a relationship to Shamonda, Aino and Akabane viruses, which do not cause risk for humans either. But it still remains unclear when and how the Schmallenberg virus had reached Europe. This is one of the important questions that needs to be answered to prevent new infections and to exclude newly emerging viruses. In the given data, a decrease in the number of cases in NRW is obvious, while the number of infected animals in new areas is increasing. This seems to be in strong correlation with the immunization of previously infected animals and the effect of the season (ACKERMANN; 2012).

If, at a certain stage of gestation (probably by the analogy to Akabane virus in sheep between 28 and 36 days and in cattle between days 75 and 110) the virus infects the fetus, severe damages may be the consequence. Besides abortions and mummified fetuses especially early or stillbirths and weak, malformed newborn lambs are typical. Similar could occur in calves.

The most common abnormalities are severe arthrogryposis (joint stiffness, tendon shortening), torticollis (strong overstretched neck) and hydranencephaly (absence of brain structures and replacement with spinal fluid, hydrocephalus). The central nervous system may be seriously affected. Overall, the clinical picture is similar to that of an infection with Akabane virus. The leading symptoms induced by the viruses of the Simbu sero-group are malformations such as "arthrogryposis-hydranencephaly syndrome" (AHS) (DOCEUL et al.; 2012).

The question is how efficiently animals can be protected against an infection and whether the spread of the viruses can be prevented- the genesis of this virus seems to be the crucial point. Obviously, the time of infection of a mother animal is of main importance to the damages resulting in the embryo.

It could be proved that infections of the mother in an early stage of gestation leads to damages of the central nervous system of the embryo while an infection of the dam at a later stage of the pregnancy will infect the damage of skeletal and muscular system of the embryos.

Vaccination might be the best solution to prevent infections like the Schmallenberg virus. But it must be taken into consideration that not only the benefits are promising for the farmers but also they need to carry the costs. It is also a problem that it is not known how the virus had reached Europe and a newly emerging virus could enter the same way in the future (ACKERMANN; 2012).

Another question is how the virus behaves in wild animals. Antibodies against the virus in alpaca, bison, deer, red deer and mouflon have been reported (LUTZ; 2012). The impact of the infection in wild animals is not yet predictable. It is important that Hunters pay attention to malformed fawns and calves, especially in areas where the virus has been detected in cattle, sheep or goats. Deformed newborns should be reported and supplied for clinical examination to the appropriate diagnostic laboratories.

At the economical point of view, it is necessary to create sensitive test in order to prevent high losses at the market. SBV is a good example for fast acting and reacting of professional institutes but it also illuminate the threat of newly emerging viruses.

7. Conclusion

Due to factors like climate change and increased globalization, an outbreak of an “exotic” vector-borne disease in Europe, as it happened with SBV, can never be excluded. It is of highest importance to sensitize the system of efficient identification and notification of emerging diseases to control such situations in the future.

8. Summary

The Schmallenberg virus, firstly detected in 2011 in a cattle herd close to Schmallenberg (North-Rhine-Westphalia, Germany), is a newly emerged Orthobunya virus (family Bunyaviridae) that is transmitted by blood-sucking insects and causes mild clinical symptoms in adult cows. Affected ruminants abort fetuses with serious congenital malformations such as athrogryphosis, brachygnathia inferior, torticollis, kyphosis, lordosis, scoliosis, muscle hypoplasia, cerebellar and cerebral hypoplasia, hydrancephaly, porencephaly, hydrocephalus, and micromyelia. The first vaccines have been developed but are still in the testing period.

Based on epidemiological data from the European Food Safety Authority (EFSA), SBV infections between August 1, 2011 and April 30, 2013 were analysed. Three ranking models (geographical, seasonal and projection model) were used to grade SBV infections regarding to animal welfare and production, such as service, milk yield and dystocia.

The diagnosis of SBV infection lays on exclusion of other viruses causing similar diseases, recognition of clinical signs and the use of a commercial ELISA kits for the detection of anti-Schmallenberg virus antibodies in serum and plasma as well as on direct virus determination from aborted fetuses, newborns and placenta by RT- PCR.

Histology showed astrogilosis and microgliosis in the central nervous system of infected bovine and ovine newborns. In some cases myofibrillar hypoplasia in the skeletal muscle was found. In sheep, lymphohistological inflammation in the central nervous system and glial nodules in the hippocampus and mesencephalon were shown.

Fetal malformations during gestation caused severe consequences on the reproductive performance in infected herds. A transplancental infection in the tetragonic determination phase causes embryonal/fetal malformation between 28 and 36 days in sheep, between 30 and 50 days in goats, and between 75 and 110 days in cattle. In case of dead bovine fetuses fetotomy must be considered. It could be proved that infections of the dam at an early stage of gestation leads to damages of the central nervous system of the embryo/fetus while later the damage of the skeletal and muscular system develops.

Due to the appearance of this new virus, further studies are needed to determine the risk of transmission of SBV and the impact of the virus on fertility in ruminants.

9. Acknowledgements

I would like to express my deep gratitude to Dr. Árpád Csaba Bajcsy, my supervisor, for his patient guidance, enthusiastic encouragement and useful critiques of this work.

I would also like to thank Dr. Ralf Jungblut (Staatliches Veterinäruntersuchungsamt; Arnsberg) and Bernward Weber, for their advice and assistance during my research.

My grateful thanks are also extended to my family and friends for their love, support and patience throughout the last five years.

10. References

Ackermann M., (2012). RNA / Bunyaviridae "Schmallenberg" Virus (SBV). *Virus Porträts / Virologisches Institut UZH [Online] [cited: April 5,2013]* http://www.vetvir.uzh.ch/teaching/vorlesungen/virologie/unterlagen/NEWSchmallenbergvirus.pdf.

Bauer B., Jandowsky A., Schein E., Mehlitz D., Clausen P.H. (2009). An appraisal of current and new techniques intended to protect bulls against Culicoides and other haematophagous nematocera: the case of Schmergow, Brandenburg, Germany. *Parasitological Research* 105; 359-365.

Beer M., Conraths F.J., van der Poel W.H. (2012). 'Schmallenberg virus' - a novel orthobunyavirus emerging in Europe. *Epidemiological Infection.* 44; 1-8.

Bilk S., Schulze C., Fischer M., Beer M., Hlinak A., Hoffmann B. (2012). Organ distribution of Schmallenberg virus RNA in malformed newborns. *Veterinarian Microbiology.* 159;236-8

Bouloy M., Pardigon N., Vialat P., Gerbaud S., Girard M. (1990). Characterization of the 5' and 3' ends of viral messenger RNAs isolated from BHK21 cells infected with Germiston virus (Bunyavirus). *Virology;* 175; 50-8.

Bréard E., Lara E., Comtet L., Viarouge C., Doceul V., Desprat A., Vitour D., Pozzi N., Cay A.B., De Regge N., Pourquier P., Schirrmeier H., Hoffmann B., Beer M., Sailleau C., Zientara S. (2013). Validation of a commercially available indirect ELISA using a nucleocapside recombinant protein for detection of Schmallenberg virus antibodies. *PLoS One*. 8; 794-8.

Bridgen A., Weber F., Fazakerley J.K., Elliott R.M. (2001). Bunyamwera bunyavirus nonstructural protein NSs is a nonessential gene product that contributes to viral pathogenesis. *Proceedings of the National Academy of Science USA;*98; 664-9.

Calisher, C. H (1996). History, classification, and taxonomy of viruses in the family Bunyaviridae. In The Bunyaviridae. Edited by Elliott RM. New York: Plenum.

Carpenter S, Mellor P.S, Torr S.J (2008). Control techniques for Culicoides biting midges and their application in the UK and northwestern Palaearctic. Medical & Veterinary Entomology, 22, 175-87.

Conraths F., Peters M., Beer M.(2012). Schmallenberg virus, a novel orthobunyavirus infection in ruminants in Europe: potential global impact and preventive measures. *New Zealand Veterinarian Journal;* 61. 63-7.

De Regge N., Deblauwe I., De Deken R., Vantieghem P., Madder M., Geysen D., Smeets F., Losson B., van den Berg T., Cay AB. (2012). Detection of Schmallenberg virus in different Culicoides spp. by real-time RT-PCR. *Transbound Emerg Dis.* 44; 471–5.

Doceul V., Lara E., Sailleau C., Belbis G., Richardson J., Bréard E., Viarouge C., Dominguez M., Hendrikx P., Calava D., Desprat A., Languille J., Comtet L., Pourquie P. (2013). Epidemiology, molecular virology and diagnostics of Schmallenberg virus, an emerging orthobunyavirus in Europe. *Veterinary Research.* [Online] http://www.veterinaryresearch.org/content/44/1/31.

Dominguez M., Hendrikx P., Zientara S., Calavas D., Jaÿ M., Touratier A., Languille J., Fediaevsky A. (2012). Preliminary estimate of Schmallenberg virus infection impact in sheep flocks - France. *The Veterinary Record.* 10: 2768–857

Ducomble T., Wilking H., Stark K., Takla A., Askar M., Schaade L. (2012). Lack of evidence for Schmallenberg virus infection in highly exposed persons, Germany, *Emerging Infectious Diseases. [Online] [cited: 7 June 2013] http://dx.doi.org/10.3201/eid1808.120533.*

Elbers A.R.W., Meiswinkel R.,Van Weezep E., Sloet van Oldruitenborgh-Oosterbaan M. M. , Kooi E. A.(2013). Schmallenberg virus in Culicoides spp. biting midges, the Netherlands, 2011. *Emerging Infectious Diseases.[Online].[cited: January 5,2013] http://dx.doi.org/10.3201/eid1901.121054*

Elliot, R.M. (1990). Molecular biology of the Bunyaviridae. *Journal of Genetic Virology*, 71. 501-22.

Elliott R. M., Blakqori G., Van Knippenberg I., Koudriakova E., LI P., Mclees A., Shi X., Szemiel A. M. (2013). Establishment of a reverse genetic system for Schmallenberg virus, a newly emerged orthobunyavirus in Europe. *Journal of General Virology.* 94, 851-9.

European Commision, (2012). Statement on the Schmallenberg Virus Situation issued by the European Commission together with the EU Member States following the working group . [Online] [Cited: March 20, 2013.] http://ec.europa.eu/food/animal/diseases/schmallenberg_virus/docs/statement_schmallenberg_17022012_en.pdf.

European Food Safety Authority, (2013). 'Schmallenberg' Virus: Analysis of the Epidemiological Data. [Online] [Cited: June 26, 2013.] http://www.efsa.europa.eu/en/supporting/doc/429e.pdf.

Garigliany M.M., Bayrou C., Kleijnen D., Cassart D., Jolly S., Linden A., Desmecht D.(2012). Schmallenberg virus: a new Shamonda/Sathuperi-like virus on the rise in Europe. *Antiviral Research* ; 95. 82-7.

Goller K. V., Höper D., Schirrmeier H., Mettenleiter T.C., Beer M. (2012). Schmallenberg virus as possible ancestor of Shamonda virus. *Emerging Infectious Diseases.* 18. 1644-1646.

Goos J. (2012). Arbeitsgemeinschaft Deutscher Rinderzüchter. *Export von Zuchtrindern und Rindersamen 2012.* [Online] [Cited: June 15, 2013.] http://www.adr-web.de/export_von_zuchtrindern_und_rindersamen_2012.html?highlight=schmallenberg&.

Gould E.A., Higgs S., Buckley A., Gritsun T.S. (2006). Potential arbovirus emergence and implications for the United Kingdom. *Emerging Infectious Diseases.*12; 549-55.

Hahn K., Habieriski A., Herder V., Wohlsein P., Peters M., Hansmann F. ,Baumgärtner W.(2013). Schmallenberg Virus in Central Nervous System of Ruminants. *Emerging Infectious Diseases;.*19: 154-5.

Harding B., Copp A. (1997). Malformations. *In: Greenfield's Neuropathology, D. Graham and P. Lantons, Eds*, 1, ,397-507.

Hartley W.J., Della-Porta A.J., Snowdon W.A., Shepherd N.C. (1977). Pathology of congenital bovine epizootic athrogryposis and hydranencephaly and its relationship to Akabane virus. *Australian Veterinary Journal,* 53, 319–325.

Herder V., Hansmann F., Wohlsein P., Baumgärtner W., Peters M., Varela M., Palmarini M. (2013). Immunophenotyping of Inflammatory Cells Associated with Schmallenberg Virus Infection of the Central Nervous System of Ruminants. *PLoS ONE.* 8;588-91

Hoffmann B., Scheuch M., Hoper D., Jungblut R., Holsteg M., Schirrmeier H.,Eschbaumer M., Goller K.V., Wernike K., Fischer M., Breithaupt A., Mettenleiter T.C., Beer M. (2012). Novel orthobunyavirus in Cattle, Europe, 2011. *Emerging Infectious Disease,* 18; 469–72.

Höper D., Wernike K.,Eschbaumer M. , Conraths F., Hoffmann B., Schirrmeier H. (2012). "Schmallenberg-Virus" - Ein neues Virus in Europa. *Deutsches Tierärzteblatt 4;*500-5.

Humphries D., Burr P. (2012). Schmallenberg virus milk antibody ELISA. *The veterinary record.* 171;511-12.

Just F., Deischl K. (2013). "Schmallenberg-Virus"(Europäisches Shamonda-like Orthobunyavirus). [Online] [Cited: March 15, 2013.] http://www.lgl.bayern.de/tiergesundheit/tierkrankheiten/virusinfektionen/schmallenberg_virus/.

Loeffen W., Quak S., De Boer-Luijtze E., Hulst M., Van Der Poel W., Bouwstra R.,Maas R. (2012). Development of a virus neutralisation test to detect antibodies against Schmallenberg virus and serological results in suspect and infected herds. *Acta Veterinaria Scandinavica* 54;44.

Lutz W, (2012). Schmallenberg-Virus: Wildtiere auch betroffen? *Landwirtschaftliches Wochenblatt Westphalen-Lippe [Online][cited: June 8, 2013] http://www.wochenblatt.com/rubriken/aktuelles/meldung/m-id/schmallenberg-virus-wildtiere-auch-betroffen/1/.*

Maxie M., Youssef S. , Brown C. ,Baker D. , Barker I. (2007). Nervous System, Alimentary System & Abortion and stillbirth in Female Genital System. . Grant Maxie. *Jubb, Kennedy & Palmer's Pathology of Domestic Animals.* s.l. : Saunders WB Co.

Mellor P.S., Kirkland P.D. (2008). *Akabane virus.* San Diego : In: Brian W. J. Mahy und Marc H. van Regenmortel (eds.): Encyclopedia of Virology, l.3; 76–80.

Metzner, M. (2012). *'Schmallenberg-Virus'-Infektion.* s.l. : Klinik für Wiederkäuer mit Ambulanz und Bestandsbetreuung am Zentrum für Klinische Tiermedizin der Tierärztlichen Fakultät, Ludwig-Maximilians-Universität München.[Online][cited: March 23,2013] http://www.rinderskript.net/skripten/b11-4.html

Pandrangi A., (2012). Etiology, pathogenesis and future prospects for developing improved vaccines against bluetongue virus: A Review. *African Journal of Environmental Science and Technology.* 7, 68-80.

Peperkamp K., Dijkman R., Van Maanen C., Vos J., Wouda W., Holzhauer M., Van Wuijckhuise L., Junker K., Greijdanus S., Roumen M. (2012). Polioencephalo- myelitis in a calf due to infection with Schmallenberg virus. *Veterinary Record ,* 170. 570.

Peters M., Köß C. (2012). Picture 2 and 3 Lamb affected by arthogryphosis (persistent flexion of the joints) and Brachygnathia inferior.[Online][cited. June 6; 2013] http://www.landwirtschaftskammer.de/landwirtschaft/tiergesundheit/hgd/schmallenberg.htm.

Plassmeyer M.L., Soldan S.S., Stachelek K.M., Roth S.M., Martin-Garcia J., Gonzalez-Scarano F.(2007). Mutagenesis of the La Crosse Virus glycoprotein supports a role for Gc (1066-1087) as the fusion peptide. *Virology* 358:. 273–82.

Reinking E., Beidler A. (2011). Jahresbericht 2011. *Friedrich-Loeffler-Institut, Bundesforschungsinstitut für Tiergesundheit.* [Online] [Cited: June 13, 2013.] http://www.fli.bund.de/fileadmin/dam_uploads/Jahresberichte/2011/Jahresbericht_2011.pdf.

Reusken C., Van Den Wijngaard C., Van Beek P., Beer M., Bouwstra R., Godeke G. J., Isken L. , Van den Kerkhof H., Van Pelt W., Van der Poel W. Reimerink J.,Schielen P.,Schmidt-Chanasit J.,Vellema P., De Vries A.,Wouters I.,Koopmans M. (2012). Lack of Evidence for Zoonotic Transmission of Schmallenberg Virus. *Emerging Infectious Diseases;* 18; 1746-54.

Roberts H.(2012). DEFRA Update 9 on Schmallenberg Virus in Northern Europe. [Online] [Cited: March 21, 2013.] http://www.defra.gov.uk/animal-diseases/files/poa-schmallenberg-update-120618.pdf.

Schatzberg S. J., Haley N.J., Barr S.C.,Parrish C., Steingold S., Summers B.A.,Delahunta A., Kornegay J. N.,Sharp N. J. (2003). Polymerase chain reaction (PCR) amplification of parvoviral DNA from the brains of dogs and cats with cerebellar hypoplasia. *Journal of Veterinarian Internal Medicine;* 17;538-44.

Schmaljohn C., Nichol S. (2007). Bunyaviridae. *Fields Virology, D. Knipe, Ed, Lippincott Williams and Wilkins, Philadelphia.5;* 1741-89.

Steukers L., Bertels G., Cay A.B., Nauwynck H.J.(2012). Schmallenberg virus: emergence of an Orthobunyavirus among ruminants in Western Europe. *Vlaams Diergeneeskundig Tijdschrift; 81.*119-27.

Summers B.A., Cummings J.F., de Lahunta A. (1995). Veterinary Neuropathology. St. Louis : Mosby-Year Book, 94 ;75–77.

Szemiel A.M., Failloux A., Elliott R.M. (2012). Role of Bunyamwera Orthobunyavirus NSs Protein in Infection of Mosquito Cells. *PLoS Neglected Tropical Diseases.* 6;.128-9.

Tarlinton R., Daly J., Dunham S., Kydd S. (2012). Scenarios for the future spread of Schmallenberg virus. *The Veterinary Record,* 170 , 245–6.

Van den Brom R., Luttikholt S.J., Lievaart-Peterson K., Peperkamp N.H., Mars M.H., Van der Poel W.H., Vellema P. (2012). Epizootic of ovine congenital malformations associated with Schmallenberg virus infection. *Tijdschrift voor Diergeneeskunde,* 137;106–11.

Varela M., Schnettler E., Caporale M., Murgia C., Barry G. (2013). Schmallenberg Virus Pathogenesis, Tropism and Interaction with the Innate Immune System of the Host. *PLOS.*9;133 -5

Viral Zone, (2012). Model of bunyavirus. [Online] [Cited: June 6, 2012.] http://viralzone.expasy.org/all_by_species/82.html.

Walter C.T., Barr J.N. (2011). Recent advances in the molecular and cellular biology. *Journal of Genetic Virology.*92; 2467–84.

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

White M.E., Whitlock R.H., Lahunta A. (1975). A cerebellar abiotrophy of calves. *Cornell Vet.* 65*;* 476–491.

Wilson A, Darpel K, Mellor P.S. (2008). PLoS Biology. *Picture Newborn calf with SBV infection.* 6;.210.

Yanase T., Aizawa M., Kato T., Yamakawa M., Shirafuji H., Tsuda T. (2010). Genetic characterization of Aino and Peaton virus field isolates reveals a genetic reassortment between these viruses in nature. Virus Research;153:1–7.

Yanase T., Maeda K., Kato T., Nyuta S., Kamata H., Yamakawa M., Tsuda T. (2005). The resurgence of Shamonda virus, an African Simbu group virus of the genus Orthobunyavirus, in Japan. *Archives of Virology ,* 150. 361–9.

Yanase T., Yoshida K., Ohashi S., Kato T., Tsuda T. (2003). Sequence analysis of the medium RNA segment of three Simbu serogroup viruses, Akabane, Aino,and Peaton viruses. *Virus Research,* 93; 63-69.

Zachery J., McGavin M. (2007). *Pathologic Basis of Veterinary Disease.Nervous System, St. Louis, Missouri, : Mosby Elsevier*. 833- 971.

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Number of files submitted: 2

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