

University of Veterinary Medicine Hannover

**Investigations on the distribution and transmission of
Schmallenberg virus in sheep and goat flocks**

Thesis

Submitted in partial fulfilment of the requirements for the degree
-Doctor of Veterinary Medicine-
Doctor medicinae veterinariae
(Dr. med. vet.)

by
Carina Monika Helmer
Ibbenbüren

Hannover 2013

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To my family and friends

Parts of this work have already been published in the following journals:

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Survey of Schmallenberg virus (SBV) infection in German goat flocks,
Epidemiology and Infection 2013, 141: 2335-2345.

Helmer C, Eibach R, Tegtmeyer PC, Humann-Ziehank E, Runge M, Ganter M.
Serosurvey of Schmallenberg virus infections in sheep and goat flocks in Lower Saxony,
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Schmallenberg-Virus Infektionen

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Schmallenberg-Virus – a new emerging disease in Germany

R. Eibach, C. Helmer, P. C. Tegtmeyer, E. Humann-Ziehank, M. Ganter

Oral presentation

8th International Sheep Veterinary Congress, Rotorua, New Zealand, 18.-22. February 2013
Monitoring of Schmallenberg-Virus (SBV) Infections in German goat flocks

C. Helmer, R. Eibach, P. C. Tegtmeyer, E. Humann-Ziehank, M. Ganter

Oral presentation

8th International Sheep Veterinary Congress, Rotorua, New Zealand, 18.-22. February 2013

Epidemiologische Erhebungen zur Verbreitung des Schmallenberg Virus bei Schafen und Ziegen

C. Helmer, R. Eibach, P.C. Tegtmeyer, E. Humann-Ziehank, M. Ganter

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Aktueller Stand zur Verbreitung der Schmallenberg-Virus Infektion

C. Helmer, R. Eibach, P.C. Tegtmeyer, E. Humann-Ziehank, M. Ganter

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List of abbreviations

%	per cent
~	about/ approximately
<	less than
>	greater than
≤	less or equal
≥	greater or equal
®	registered sign
°C	degree centigrade
µl	microliter
A-H-syndrome	arthrogryposis-hydranencephalia-syndrome
Apr	April
Aug	August
BLE	Bundesanstalt für Landwirtschaft und Ernährung (Federal Office for Agriculture and Food)
BMELV	Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz (Federal Ministry of Food, Agriculture and Consumer Protection)
BTV	Bluetongue virus
C.	Culicoides
C.H.	Carina Helmer
CVUA	Chemisches und Veterinäruntersuchungsamt (Chemical and Veterinary Investigation Office)
Dec	December
DFNVC	Deutsches Forschungsnetz video conference (Video Conference Service of the German Research Network)
DNA	Deoxyribonucleic acid
EDTA	ethylene diamine tetraacetic acid
EFSA	European Food Safety Authority
ELISA	enzyme-linked immunosorbent assay
et al.	et alii
Feb	February
Fig.	Figure
FLI	Friedrich-Loeffler-Institute
g	gravity
GmbH	Gesellschaft mit beschränkter Haftung (company with limited liability)
HE	Hesse
i.e.	id est
Inc.	Incorporated

- List of abbreviations -

Jan	January
Jul	July
Jun	June
km	kilometer
L	large
LS	Lower Saxony
M	medium
Mar	March
max	maximum
min	minimum
mRNA	messenger ribonucleic acid
MVP	Mecklenburg Western Pomerania
neg.	negative
Nov	November
NRW	North Rhine-Westphalia
Oct	October
p.i.	post infectionem
PCR	polymerase chain reaction
pos.	positive
p-value	probability value
RNA	ribonucleic acid
rRT-PCR	real-time reverse transcriptase polymerase chain reaction
RT-PCR	real-time polymerase chain reaction
S	small
S/P	sample/postive
SA	Saxony-Anhalt
SAS	Statistical Analysis Systems
SBV	Schmallenberg virus
SH	Schleswig-Holstein
spp.	subspecies
UK	United Kingdom
vs.	versus

1. Introduction

In early autumn 2011 a new emerging disease was reported from dairy cattle farmers from the Netherlands and Germany including mild fever, therapy-resistant diarrhoea and a reduction in milk yield (MUSKENS et al. 2012). Thereafter the Friedrich-Loeffler-Institute (FLI, Federal Institute for Animal Research, Germany) traced a new virus utilizing a meta-genomic approach with next generation sequencing after classical bovine endemic and emerging viruses such as Bluetongue virus (BTV), Foot and Mouth disease virus, Bovine viral diarrhoea virus, Border disease virus, Bovine herpes virus-1, Epizootic haemorrhagic disease virus, Rift Valley fever virus and Bovine ephemeral fever virus had been excluded as possible causes of the recorded occurrences (HOFFMANN et al. 2012). According to its place of first detection, a city called Schmallenberg located in the Sauerland, North Rhine-Westphalia, Germany, the virus was provisionally named Schmallenberg virus (SBV). SBV belongs to the family Bunyaviridae, genus Orthobunyaviruses and is identified to be a single stranded negative-sense RNA virus. Its genome is divided into three segments: small (S), medium (M) and large (L) (HOFFMANN et al. 2012). Phylogenetic analysis revealed that SBV is a member of the Simbu serogroup and is closely related to Akabane, Aino and Shamonda virus. Investigations of GOLLER et al. (2012) indicate that SBV belongs to the species Sathuperi virus and is suggested to be a possible ancestor of the reassortant Shamonda virus. All of these viruses are known to primary infect ruminants (HOFFMANN et al. 2012) and may induce malformations in newborns if a susceptible mother animal is infected during early pregnancy (PARSONSON and MCPHEE 1985). Orthobunyaviruses are arthropod-borne viruses and are mainly transmitted by mosquitoes and biting midges, first and foremost *Culicoides (C.) spp.* (HOFFMANN et al. 2012).

Hitherto, Bunyaviruses are known to occur in Africa, Asia, the Middle East and Australia but were so far never described in Central Europe before (LIEVAART-PETERSON et al. 2012; SAEED et al. 2001). From September 2011 until April 2013 more than 8000 holdings with laboratory confirmed SBV cases have been recorded in twenty European member states (Austria, Belgium, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Hungary, Germany, Ireland, Italy, Latvia, Luxembourg, the Netherlands, Poland, Slovenia, Spain,

Sweden and United Kingdom) and two countries in the European Free Trade Association area (Switzerland and Norway) (ANONYMOUS a 2013) (Fig. 1). Moreover antibodies against SBV were found in Turkey (AZKUR et al. 2013). At present, 2478 SBV cases have been proven in Germany by RT-PCR with 1459 cattle herds, 969 sheep flocks and 50 goat flocks being positive with a high estimate of unreported cases (ANONYMOUS b 2013). In March 2012 SBV was given the status of a reportable animal disease in Germany and the Netherlands. On this basis the detection of the infectious agent (virus) by RT-PCR in blood samples or organs have to be reported to the responsible Veterinary Inspection Offices for statistical reasons.

Vector-borne diseases have long been considered of minor importance in central and northern Europe resulting in vanishing interest in vectors and vector research as almost no vector-borne disease of epidemiological relevance had occurred for decades (KAMPEN and WERNER 2010). Since the advent of the BTV epidemic in 2006 this attitude has changed. Due to continuing globalization and climate shifts even countries with a moderate European climate are now at risk of vector-borne disease outbreaks as BTV and SBV. The way in which SBV has entered Europe has remained obscure until now. The following routes of entry are, however, possible and need to be considered:

1. Introduction by viraemic vertebrates: This possibility is rather unlikely because of the very short viraemic period of SBV which only lasts for 2 to 5 days p.i. (HOFFMAN et al. 2012). Nevertheless it must be taken into consideration that viraemic ruminants or game and wild animals may have served as infection sources for indigenous biting midges.
2. Introduction by virus-carrying midges: Biting midges can be transported by the wind over short-way-distances as well as over long-way-distances. Several studies report on wind driven spreads of *Culcioides spp.* over sea of up to 700 km. Long-distance spread over land follows a so called hopping pattern with distances of 35-85 km (HENDRICKX et al. 2008, DUCHEYNE et al. 2007; SELLERS and PEDGLEY

1985; SELLERS 1992). During the BTV outbreak 2006 several models have been released to estimate the windborne dispersal from Africa to Europe (SEDDA et al. 2012; HENDRICKX et al. 2008).

Another way of entry might be by an accidental import of virus-carrying biting midges into Europe by global transports (i.e. maritime or air traffic). Cargo ships and aircrafts transporting flowers, plants, fruits, vegetables and livestock from overseas to big European seaports (i.e. Rotterdam, The Netherlands; Hamburg, Germany) and airports (Schiphol, Amsterdam, The Netherlands; Frankfurt Rhein-Main, Frankfurt, Germany) might have had SBV-infected vectors as *C. spp.* and mosquitoes on board. The possibility of such a transmission route was already proven for China, as NIE et al. (2005) have demonstrated that living biting midges were brought to China from overseas by ship. Similar to the development described by NIE et al. (2005) the imported vectors hit excellent climate conditions and a naïve ruminant population in central and northern Europe, thus being able to reproduce very rapidly and provoking a large SBV outbreak in 2011/2012 in Germany, the Netherlands and Belgium. Since then the epidemic spread to almost all European countries in only two years' time (Fig. 1).

LEASK et al. (2013) stated that deformities in lambs were observed in several sheep flocks in South Africa in 2006 and 2008. As BTV, Wesselbron disease, Rift Valley fever, Akabane disease and Middelburg virus could be ruled out as causative agents at that time the cause was thought to be a new strain of Akabane virus as for example SBV. Unfortunately the samples taken in 2006 and 2008 are not available anymore so that a confirmation of SBV-infection is not possible. However, the recent discovery of SBV in central Europe raises the question whether SBV has been present in South Africa during the last years without being diagnosed and identified and was now introduced into Europe due to global transports.

Furthermore, it is conspicuous that SBV was introduced to central and northern Europe in almost exactly the same way as BTV-8 did in the 2006/2007 epidemic. BTV-8 is another arthropod-borne-virus affecting ruminants and was transmitted to Europe from the sub-Saharan African continent. First outbreaks of BTV were also

reported from the border triangle Belgium, the Netherlands, Germany and Northern France and made its way through Europe very quickly. Although the route of BTV entry was not detected thus far speculations on how BTV entered Europe are very similar to the above mentioned hypotheses for SBV (KAMPEN and WERNER 2010).

3. Introduction by contaminated biological products: Another possibility of SBV incursion into Europe would be the import of SBV contaminated products as sera, semen, ova and embryos but there are no indices for such a route of entry so far. It is important to point out, however, that the FLI recently did detect SBV in the semen of bulls by RT-PCR (HOFFMANN et al. 2013).

Based on the pathogenesis of Akabane virus it can be assumed that a direct transmission from animal-to-animal is rather unlikely. Symptoms of adult animals, if they show any, are mild fever, diarrhoea, languor and a reduction of milk yield. Vertical transmissions during gestation are possible and can induce congenital malformations if a naïve mother animal becomes infected during a vulnerable period in early gestation. In small ruminants an infection from day 1 until day 28 of pregnancy can lead to increases in embryonic and foetal death, as well as increased proportions of females returning to oestrus. Furthermore delivery of small offspring and decreased fertility in the following oestrus cycle are described. If the ram was removed early from the flock these occurrences can result in an increased proportion of barren mother animals. If the ewe/doe gets infected from day 28 until day 56 of gestation malformations, mummifications and abortions are observed in newborns presuming that the second month of gestation is the most hazardous period for the unborn offspring. After day 56 of pregnancy the foetuses are supposed to fight the virus with its matured immune system although stillbirth, abortion and mummification can be observed in individual instances (ANONYMOUS 2009). Thus far there is no evidence to refute the assumption that SBV-infections induce a long-term immunity in affected animals so that clinical signs can only be observed in firstly infected ruminants (ANONYMOUS a 2013). Whether the pathogenesis of Akabane virus can be entirely transferred to SBV needs to be confirmed in further studies.

First cases of SBV-infections were reported almost simultaneously from the Netherlands, Belgium and the north-western parts of Germany. Primarily dairy cattle farmers reported therapy-resistant diarrhoea, mild fever and a reduction in milk yield to animal health services of the affected countries (MUSKENS et al. 2012). Since SBV is a viral infection no therapy was possible and the symptoms abated after some weeks. The economic losses of the affected dairy cattle farms were rather low. With the beginning of the lambing season 2011/2012, which started at the end of November 2011, first reports on malformed and stillborn lambs were recorded in sheep and goats in the Netherlands and Germany. Typical congenital malformations can be summarised by the arthrogryposis-hydranencephaly-syndrome (A-H-syndrome), reflecting in stiffening of the joints and distension of the cranium, curvatures of the spine (scoliosis, lordosis, kyphosis and torticollis) and brachignathia or agnathia. Frequent patho-morphological findings were malformations of the central nervous system as hydranencephaly, anencephaly, porencephaly, cerebellar hypoplasia and brain stem hypoplasia besides several musculoskeletal and vertebral malformations (HERDER et al. 2012; VAN DEN BROM et al. 2012) which are most likely a result of failures in the development of the central nervous system as true skeletal defects could not be observed in malformed neonates. VARELA et al. (2013) developed a mouse model to study SBV-infection and found that SBV replicates in neurons where it induces cerebral malacia and vacuolation of the cerebral cortex. Moreover they detected high levels of SBV antigens in the neurons of the gray matter of brain and spinal cord of naturally infected lambs and calves, strongly suggesting that neurons are the predominant target cells of this new emerging virus. These findings could be confirmed by investigations of HAHN et al. (2013) which found SBV mRNA in neurons of the cerebrum, cerebellum, brain stem, medulla oblongata, and spinal cord whereas no SBV mRNA was found in any peripheral organ. This again proves the hypothesis that muscular hypoplasia and skeletal defects in malformed neonates are mainly secondary to central nervous damage. The clinical signs of arthrogryposis which are characterised by stiffening of the joints and malpositions of the extremities can be explained by an abnormal foetal development of the motor neurons of the ventral horn of the spinal cord. These disorders cause an inadequate development of the muscular system with a subsidiary neurogenic muscle atrophy followed by ankylosis of the joints.

As the new emerging virus encountered a naïve ruminant population in central and northern Europe which has never previously been affected by Orthobunyavirus infections (SAEED et al. 2001) a large outbreak of the new disease could be observed throughout many ruminant species resulting in partly enormous financial losses to farmers and livestock industry. Precise data on animal losses are not available yet. Many farmers only reported individual cases of SBV affected neonates but some lost more than 50 % of their lambs due to malformations, abortion and stillbirth. Thus far SBV has been detected by RT-PCR in cattle, sheep, goats, bison, deer, moose, buffalos and alpacas (ANONYMOUS a 2013). Small ruminants were clinically affected to a higher degree than cattle resulting in sharply higher economic losses. This is probably due to the seasonality in reproduction of sheep and goats which are known to be so called short-day-breeders (WALTERS 2007). In temperate regions like Europe the mating period of seasonal breeds of small ruminants starts as soon as the day length and the light quantity decrease except of some breeds which show an aseasonal sexual activity (i.e. Merino Mutton Sheep, Cameroon Sheep, Barbados Blackbelly, Dorper and Boer goats). The transition from anoestrus to cyclicity bears on an endogenous circannual basis which is controlled by the photoperiod. Due to the shortened day length hormone levels of melatonin which is released by the pineal gland (epiphysis) during the night hours increase so that the ewe/doe begins to cycle (WALTERS 2007). Hence, mating starts in late summer/autumn. All animals are mated in a short period of time ranging from some weeks up to two or three months meaning that lambing takes place mainly from December to March (under European conditions). As a SBV-infection is only dangerous for the unborn foetuses if the infection of the mother animal takes place in early pregnancy it becomes obvious why many sheep and goat flocks reported massive losses due to malformed neonates and obstructed labours with the highest number of deformed lambs and kids reported from December to February. Cattle do not exceed seasonality in reproduction (WALTERS 2007) meaning that calving is performed almost throughout the entire year. Normally only a few animals are pregnant at the same time so that an infection with SBV is of less consequence than in small ruminants.

Culicoides spp. show a seasonal distribution throughout the year. Their activity time ranges from March to November in total with activity peaks in spring (May/June) and autumn (August/September) and a decrease of population density in winter. Most *C. spp.* are

crepuscular or nocturnal, having peaks at sunrise and sunset (GONZALES et al. 2013; ANDER et al. 2012; KIEL et al. 2009). As most small ruminants are kept out on pasture day and night with only a cote for shelter against adverse weather conditions they are not protected against biting midges during their main flight time. Exceptions are dairy goat and sheep flocks which are mainly housed in closed barns all year-round or at least overnight. Since *C. spp.* have a second activity peak in autumn (August/September) (KIEL et al. 2009) coinciding exactly with mating and early pregnancy of most small ruminant breeds. Therefore many animals were affected during the vulnerable stage of early gestation (second month) resulting in large numbers of malformed newborns and high economic losses in affected goat and sheep flocks. As SBV cases were reported not only in the 2011/2012 lambing period but also in the 2012/2013 lambing period in Germany the virus must have a strategy of hibernation. Theoretically, overwintering of SBV would be possible in the arthropod vector, in the ruminant host and in both of them within a natural winter transmission cycle (KAMPEN and WERNER 2010). Again the ruminant host is unlikely for overwintering of the virus as viraemic phases are very short (2-5 days) (HOFFMANN et al. 2012). Hibernation of SBV in the arthropod vector might be possible as single specimens of *C. obsoletus*, which are supposed to be the main vectors for SBV, were observed to live more than three months at room temperature (PARKER 1950). Vertical (transovarial) transmission from infected female insects to their offspring might also be a possible way of overwintering. Studies of LOSSEN et al. (2007), BALDET et al. (2008) and GONZALES et al. (2013) revealed that winter activity of biting midges in stables is present although the population density of *C. spp.* decreases. Hence, winter transmission cycles between vertebrate hosts and arthropod vectors are likely. This hypothesis can be substantiated by the technical report of the European Food Safety Authority (ANONYMOUS a 2013) which states that the area, where cases of SBV were reported, has expanded in winter 2012 and spring 2013. Moreover, acute cases in adult ruminants could be observed in Germany in all months from November 2011 to April 2013, strongly suggesting virus circulation during the winter period (ANONYMOUS a 2013).

Two studies on zoonotic potential of SBV implemented in Germany and the Netherlands in 2012 revealed a lack of evidence for zoonotic transmission of SBV (DUCOMBLE et al. 2012; REUSKEN et al. 2012). For both studies highly exposed persons were investigated and

neither clinical illness nor serological response could be found suggesting that the public health risk for SBV is absent or extremely low.

The aim of this study was to collect data on the emergence of SBV as a new arthropod-borne disease introduced into Europe in 2011. The focus was on the epidemiology and distribution of SBV throughout small ruminant flocks in Germany which is located in the core region of the 2011/2012 epidemic. New SBV-infections have been reported in 2013 due to the spread of SBV-infections towards southern and eastern parts of Germany and an incomplete infestation of flocks having low seroprevalences for SBV antibodies. The objective of this study was to achieve an overview of the clinical symptoms which might be caused by the viral agent as well as to gain insights of possible paths of its distribution and prevention. Since SBV very recently entered Europe in 2011 further studies are needed to understand the pathogenesis of the disease on the one hand and to find the possible route of entry into central and northern Europe on the other hand. Further information on these aspects might ultimately add to the development of reliable prevention methods and the understanding of SBV-infections as a new emerging disease agitating Europe.

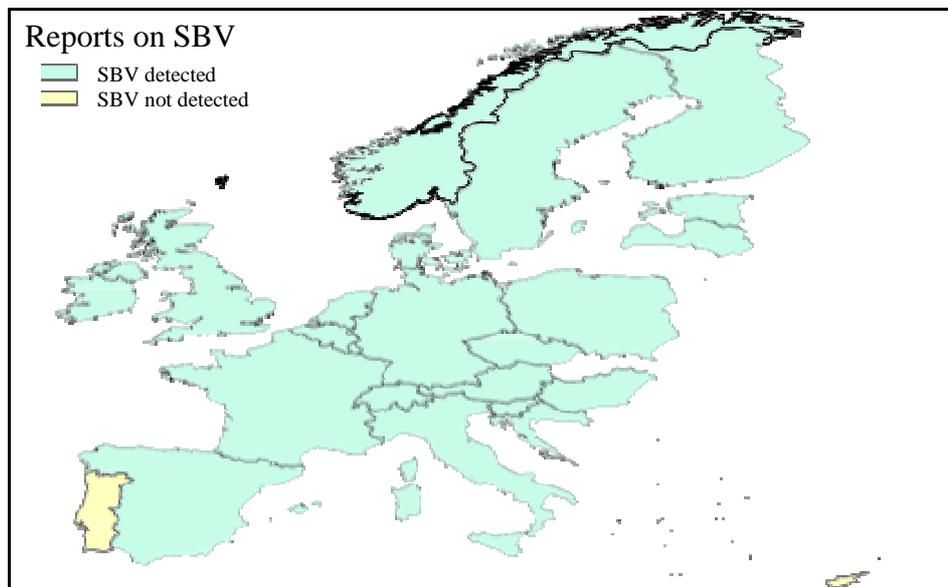


Figure 1: SBV status for European Countries (source: The European Food Safety Authority (EFSA): “Schmallenberg” virus: analysis of the epidemiological data (May 2013), Datenstand: 14.06.2013 Internet: URL: <http://www.efsa.europa.eu/en/supporting/pub/429e.htm>)

2. Manuscript I

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Survey of Schmallenberg virus (SBV) infection in German goat flocks

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Running head: SBV-infections in goats

Summary

Animal losses due to abortion and malformed offspring during the lambing period 2011/2012 amounted to 50% in ruminants in Europe. A new arthropod-borne virus, called Schmallerberg virus (SBV), was identified as cause of these losses. Blood samples were obtained from 40 goat flocks and tested for antibodies against SBV by ELISA, with 95% being seropositive. The calculated intra-herd-seroprevalence (median 36.7%, min-max 0-93.3%) was smaller than in cattle or sheep flocks. Only 25% of the farmers reported malformations in kids. Statistical analysis revealed a significantly lower risk of goats housed indoors all year-round to be infected by SBV than for goats kept outside day and night.

The low intra-herd-seroprevalence demonstrates that German goat flocks are still at risk of a SBV-infection. Therefore, they must be protected during the next lambing seasons by rescheduling the mating period, implementing indoor housing, and continuous treatment with repellents or vaccination.

Keywords: Bunyaviruses, estimating, infectious disease, prevalence of disease.

3. Manuscript II

Transboundary and Emerging Diseases

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Survey of Schmallenberg virus infections in sheep and goat flocks in Lower Saxony, Germany

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Running head: SBV-infections in small ruminants, Lower Saxony, Germany

Summary

Schmallenberg virus (SBV) infections can cause congenital musculoskeletal and vertebral malformations as well as neurological failures in fetuses of several ruminant species, if susceptible mother animals were infected during early gestation. Blood samples gained from 17 goat and 64 sheep flocks in Lower Saxony (LS), Germany (January-May 2012) which is located in the core region of the 2011/2012 epidemic were tested for antibodies against SBV by ELISA in order to detect past-exposure to SBV. A SBV-specific questionnaire was raised in all flocks. The calculated median within-herd-prevalence was 43.8% (min-max: 5.6-93.3%) for goats and 58.7% (min-max: 6.5-100%) for sheep, showing that small ruminants in LS, especially goats, are still at risk of novel SBV-infections in the following lambing seasons as not all animals have seroconverted yet. Statistical analysis revealed that goats have a significantly lower risk of SBV-infections than sheep which might be explained by different host preferences of *Culicoides* ssp. as main vectors for SBV and different housing conditions.

Keywords: Arboviruses, Emerging diseases, Veterinary epidemiology

4. General Discussion

SBV is a new emerging disease which firstly entered central and northern Europe in 2011. Since the first outbreaks were recorded in the border triangle composed of the Netherlands, Belgium and Germany the epidemic reached almost all European countries except Portugal and Cyprus (ANONYMOUS a 2013). In only two years` time the virus caused partially massive economic losses to farmers and livestock industry. Losses were primarily caused by malformed and stillborn offspring, abortions and losses of mother animals due to increased numbers of dystocia. Diarrhoea and reduction in milk yield were mainly observed in dairy cattle herds and might also result in financial losses although it must be admitted that clinical symptoms in adult animals are rather mild or even absent at all.

The aim of this study was to describe and understand the unexpected and unforeseen emerge of SBV as a new arthropod-borne disease in central and northern Europe. The BTV epidemic in 2006/2007 impressively proved that biting midges are able to survive and reproduce under moderate European conditions. Nevertheless, neither anybody expected nor was prepared for a new vector-borne virus entering and running over Europe that rapidly. This work was done in order to learn more about the epidemiology and distribution of this new viral agent throughout Germany which is, after the BTV epidemic, again one of the first countries affected by SBV and located in the core region of the 2011/2012 epidemic. Furthermore, a questionnaire was established in order to gain an overview of the clinical symptoms that might be caused by the virus and in order to possibly find reliable methods of prevention against SBV-infections.

The first part of this study (manuscript I) describes the epidemiology and distribution of SBV among 40 randomly selected goat flocks located in the six most SBV affected federal states of Germany (North-West Germany) during the 2011/2012 epidemic. From January until May 2012 1065 blood samples were obtained from adult female goats (>1 year) in order to detect antibodies against SBV by ELISA and then calculate the post exposure.

Due to the very rapid expansion of the disease the initially planned case-control-study with a control flock characterized to be negative for SBV antibodies needed to be converted into an epidemiological study performed under field conditions.

According to the Federal Statistical Office of Germany a total of 149,936 goats housed on 11,219 farms were kept in Germany in March 2010 (ANONYMOUS 2010). Thereof 75,544 goats were kept for breeding (about 50%). The average flock size is 13.4 goats per farm. About 10,000 goat farms in Germany are merely keeping one to 19 animals per flock (as hobby breeding animals or pet animals). The essential number of animals per flock which needed to be tested to detect the presence of antibodies to SBV at expected seroprevalence of 20% and 95% level of confidence was $n \geq 14$. Of the remaining 1272 goat flocks large enough to be surveyed 288 goat flocks were located in the six federal states tested for the study (Hesse, Lower Saxony, Mecklenburg Western-Pomerania, North Rhine-Westphalia, Saxony-Anhalt and Schleswig-Holstein). Thereof 40 goat flocks were randomly selected in the six most SBV-affected federal states of Germany and tested for antibodies against SBV by ELISA. Regarding the epidemic development it needs to be pointed out that at the beginning (December 2011/ January 2012) the federal states Lower Saxony, North Rhine-Westphalia and Schleswig-Holstein were the only to report SBV-infections in goats. Therefore it was planned to test ten goat flocks in Lower Saxony, North Rhine-Westphalia and Schleswig-Holstein respectively (30 flocks in total, five case and five control farms for each federal state, with a control farm characterised as a flock with no serological or virological proof of SBV-infection). Analysing the first serological results it became obvious that there were no goat flocks tested seronegative for SBV in Lower Saxony and North Rhine-Westphalia. Due to this epidemic development the planned case-control-study was converted to an epidemiological survey on SBV-infections in goats for the most SBV-affected federal states of Germany (state January 2012 until the beginning of June 2012). During the incredibly fast progression of SBV-infections, Hesse, Mecklenburg Western-Pomerania and Saxony-Anhalt soon followed in reporting cases of malformed goat kids, possibly caused by SBV-infections. Therefore, these federal states were also quickly taken into consideration for the survey. By that procedure the fast epidemiological development has been observed and analysed additionally.

As described in manuscript I, 95% of the 40 goat flocks tested for the survey had antibodies against SBV. Nevertheless it must be admitted that the estimated median within-herd prevalence was only 36.7%, meaning that on herd-basis almost all flocks were positive for SBV but on within-herd-basis only a few animals per flocks had built antibodies against the virus. Manuscript II underlines these findings by showing that goat flocks in Lower Saxony (LS) are less affected by SBV-infections than sheep flocks. The median within-herd prevalence for goats in LS was 43.8% and 58.7% for sheep. The difference between these two small ruminant species is significant (P value: 0.0058). In general it can be supposed that goats are less affected by SBV-infection than sheep and cattle shown by lesser median within-herd prevalences. The reason for these differences might be explained as follows:

1. Different host preferences of the vectors: Most *Culicoides spp.* prefer to feed on cattle if present (NINIO et al. 2011; LASSEN et al. 2011; LASSEN et al. 2012; BARTSCH et al. 2009). The thick fleece of sheep makes the reachable surface for midge bites fairly small as only non-woollen or little woollen areas of the skin such as the udder, the inner shank and thigh, the interdigital skin, the head and the bottom side of the tail are approachable for bites of *C. spp.*. Possibly the species-specific odour of goats might be a reason for the lower attractiveness for biting midges compared to cattle and sheep.
2. Different housing conditions and types of utilization: Most small ruminant flocks in Germany are kept extensively out on pasture day and night. Main reasons for sheep and goat farming are landscape protection, meat production and breeding. The only time of the year where animals are stabled is during the lambing period. Exceptions are dairy sheep and goat flocks which are mainly kept indoors on deep litter all year-round or at least during the night. As manuscript I revealed, permanently housed goat flocks were considerably less affected by SBV-infections than goat flocks kept permanently outdoors meaning that housing of animals, at least during the night time, is an adequate method to prevent SBV-infections in small ruminants. In comparison to most small ruminant flocks German cattle herds are mainly kept indoors all year-long on slatted floors with cesspools for the storage of dung below. Although most cattle is

permanently housed throughout the entire year the seroprevalences of cattle is much higher than for sheep and goats with 72.5% in the Netherlands (ELBERS et al. 2012) and 90.8% in Belgium (GARIGLIANY et al. 2012). This is most probably due to the storage of the very liquid and nutrient-rich slurry which provides perfect conditions for the reproduction and development of *C. spp.*. Several studies outlined that *C. spp.* breed in cattle manure and may even use it for hibernation (MEISWINKEL et al. 2008; ZIMMER et al. 2008). In contrast to that no larval stages of biting midges could be found in pure sheep droppings or soil inside sheep barns (GONZALES et al. 2013).

3. Different stabilities of anti-SBV antibodies: A third possibility why goats seem to be less affected by SBV-infections than other ruminant species is that the anti-SBV antibodies they built might disintegrate more quickly than those of sheep and cattle. This hypothesis can be substantiated by the fact that during a long-time monitoring for SBV antibody activity on a dairy goat farm in North Rhine-Westphalia, Germany SBV antibodies induced by natural infection started to decrease rapidly after seroconversion in many of the animals tested for the study (unpublished data, Clinic for Swine and Small Ruminants, University of Veterinary Medicine Hannover, Foundation). Further investigations are needed to clarify these findings. If it would be proved that SBV-infections in goats do not induce a long-term immunity against the viral infection it must be considered that reappearing SBV-infections might be possible in one and the same animal. This would mean that the hypothesis of animals being protected against SBV after first contact must be modified for goats. Nevertheless there is no evidence to refute the assumption that SBV-infections induce a long-term immunity in affected animals thus far so that it must be admitted that clinical signs can only be observed in firstly infected ruminants (ANONYMOUS a 2013).

There is little known about behaviour and biology of biting midges in general, even after the recent outbreak of BTV seven years ago which should have given rise to more detailed studies on *C. spp.*. As already mentioned before, vector-borne diseases have long been considered of minor importance in central and northern Europe resulting in vanishing interest

in vectors and vector research as almost no vector-borne disease of epidemiological relevance had occurred for decades (KAMPEN and WERNER 2010). Some studies on distribution, breeding and developmental sites, host-preferences and biting behaviour of *C. spp.* have been initiated since the BTV outbreak in 2006/2007, but nevertheless there is almost no literature available concerning *C. spp.*. This results in poor knowledge of the biology of biting midges which would be of great importance for surveillance programs and prevention methods.

Both implemented studies reveal that German sheep and especially goat flocks are still at risk of novel SBV-infections due to incomplete infestations of flocks in already affected areas and the expansion of SBV outbreaks from affected areas to southern and eastern parts of Germany. A 100% protection against SBV is not possible at the moment as there is no licensed vaccine available in Germany yet. Several pharmaceutical agencies are working on a protective vaccine against SBV. Just recently the Veterinary Medicines Directorate has granted a provisional authorisation of an inactivated SBV vaccine (Bovilis SBV®, MSD Animal Health) for the United Kingdom (UK) market meaning that UK farmers will be the first in Europe to have access to a licensed SBV vaccine which will be available across the summer period 2013 (ANONYMOUS c 2013). Possible strategies, to at least reduce the risk of new SBV-infection in Germany as long as no vaccine is licensed, are permanent animal housing (1), strict and continuous treatment with pharmaceuticals against external parasites (2) and rescheduling the mating period (3).

1. Permanent animal housing: As manuscript I describes permanent housing might be an option to protect small ruminants against SBV-infections. Permanently housed small ruminants are normally kept on deep litter. Sheep and goat droppings are very arid and firm and therefore do not provide optimal breeding and developmental sites for biting midges in contrast to cattle. It might be assumed that *C. spp.* do not survive inside sheep and goat stables very long due to missing humidity. GONZALES et al. (2013) sustain this theory by not detecting any larval stages of biting midges in sheep droppings or soil inside sheep stables. These results can be transmitted to goats as sheep and goat droppings and soils are similarly structured. Insect traps and

insecticide impregnated nets might be helpful to further decrease the number of midges inside sheep and goat stables.

2. Treatment against external parasites: A strict and continuous treatment with repellents could reduce the risk of SBV-infections although it must be admitted that the duration of effect is debated controversial as manuscript I outlines. Typical pharmaceutical agents used by many shepherds in Germany are Butox 7.5 Pour-on® (Deltamethrin, Intervet Deutschland GmbH, Unterschleißheim, Germany) and Sebacil 50%® (Phoxim, Bayer HealthCare, Bayer Vital GmbH, Leverkusen, Germany). The active agent of Butox 7.5 Pour-on® is deltamethrin which is a pyrethroid ester insecticide. Deltamethrin is a neurotoxin which induces paralysis and convulsions resulting in death of the insects after absorption. The active agent of Sebacil 50%® is Phoxim which is an organophosphate insecticide. It inhibits the acetylcholine esterase and also operates as a neurotoxin. The problem with these agents against external parasites is that *C. spp.* are still able to bite and possibly transmit the virus before they are killed so that SBV-infections might still be possible although the animals were correctly treated with repellents. Another alternative for the treatment against biting midges might be neem oil, which is a vegetable oil obtained from the seed of the neem tree and licensed as biocide in Germany. The active agent of neem oil is Azadirachtin which inhibits the synthesis of chitin. Thus far it is unclear whether neem oil reliably bars *Culicoides* midges from biting. Several feed manufacturer advertise and announce that mineral or concentrated feed enriched with garlic might also be an option to prevent insects as *C. spp.* from biting.
3. Rescheduling of the mating period: By rescheduling the mating period to the colder months of the year the risk of intrauterine SBV-infections might also be reduced as *C. spp.* show a seasonal activity throughout the year with a very small population density in winter. However, it must be admitted that KIEL et al. (2009) found small numbers of *C. spp.*, especially members of the *C. obsoletus* complex, present in the coldest winter months (December-February). Another problem is due to economic reasons. Many farmers are bound to seasonal marketing strategies. In Germany the

high season for lamb meat thrives around Easter time. By rescheduling the mating period lambing would start in late winter/early spring so that farmers could not manage to fatten their lambs in time.

In late spring 2013 a fatal flood catastrophe hit large parts of Germany. Many rivers were overflowing several thousand square kilometres. These occurrences provide perfect conditions for a rapid reproduction and development of *C. spp.* during the now following warm summer period. It can be assumed that a considerable mosquito and biting midges plague will follow the flood catastrophe. Consequential effects might be high numbers of new SBV-infections during the summer and autumn months resulting in malformed newborns in the next lambing period 2013/2014 if mating is scheduled at this time of year. Farmers located in areas which were affected by the flood catastrophe should be aware of this risk and therefore should try to protect their animals against biting midges at least during the hazardous time of early gestation.

Congenital malformations can be caused by several teratogenic agents of toxic, physical or infectious nature as well as by genetic defects (GANTER 2013). Hence, differential diagnoses to possible SBV-infections are manifold and not all malformed newborns must be caused by SBV. Teratogenic viruses which might induce malformations in newborn small ruminants besides SBV belong to the families Bunyaviridae (Rift-Valley virus, Akabane virus, Aino virus, Shamonda virus and Cache-Valley virus), Reoviridae (Bluetongue virus) and Flaviviridae (Border disease virus, Bovine virus diarrhoea virus, Wesselbron virus). Genetic factors are difficult to diagnose as there are often only individual animals of a flock affected. Entropion, microphthalmia and brachygnathia are the most observed genetic malformations in sheep and goats and occur worldwide. Corridale sheep show a symptom complex including congenital malformations reflected in A-H-syndrome due to an autosomal recessive disorder. Suffolk and Hampshire sheep also show a genetic disease called Spider-lamb-syndrome which might also cause clinical symptoms that might be similar to malformations caused by SBV. Congenital malformations can also be caused by chemical and toxic agents (i.e. benzimidazoles and poisonous plants as spotted hemlock and tobacco) as well as by mineral

and vitamin deficiencies (iodine, copper, manganese, vitamin A, vitamin D). Furthermore beta and gamma radiation can physically induce congenital malformations (GANTER 2013).

As manuscript II revealed SBV isolation from brain tissue samples of affected lambs was often unsuccessful and RT-PCR results may be negative despite evident clinical and pathomorphological signs of A-H-syndrome. VAN DER POEL (2012) found that only 29% of brain tissue samples of lambs and 14% of brain tissue samples of calves showing typical signs of A-H-syndrome were tested positive for SBV by RT-PCR. This is probably due to the long time between assumed SBV-infection in early gestation and time of examination of affected newborns after birth. In the second part of gestation the foetus becomes immunocompetent, producing specific neutralising antibodies which might explain the elimination of SBV from their tissues. Thus, receiving a reliable diagnosis of SBV-infection is a problem. In many cases we have to rely on clinical, pathomorphological and histopathological findings which might be typical for SBV-infection but not pathognomic.

Lessons to be learned from the BTV and SBV epidemic in Europe are to invest in long-term entomological studies on indigenous but also on exotic arthropod vectors in order to identify potential vectors and acquire more detailed knowledge on their biting behaviour and biology. This knowledge could help to avoid and manage outbreaks of new emerging arthropod-borne diseases as SBV in a much more efficient way. More specialists in the field of entomology should be trained in order to be competent contact persons in the conceivable event of the emergence of a new vector-borne disease. Moreover we should be aware of the danger that originates from the more and more increasing globalization and climate change. Surveillance programs of indigenous and exotic arthropod vectors should be encouraged and implemented after two vector borne epidemics (BTV and SBV) running over Europe in the recent years. Furthermore, it would be rational to control goods and animals not only for pathogenic agents but also for brought in insects and vermin as the increasing globalization and climate warming probably entail new unusual diseases for European conditions.

In summary, this study is able to show that German sheep and goat flocks are still at risk of new SBV-infections due to incomplete infestations of flocks. Hence, these occurrences might

contribute to establish an enzootic SBV situation in central and northern Europe. Especially the flood catastrophe 2013 with the assumed mosquito and biting midge plague enhances the risk of SBV-infections in the next lambing season due to intrauterine SBV-infections of the unborn neonates during the warm summer and early autumn months if mating is scheduled at this time of year.

Further studies will be needed to find out more about the pathogenesis of the virus and to ascertain the so far assumed hypothesis of long-term immunity in adults. Moreover investigations concerning the route of entry of SBV into Europe should be implemented in order to possibly reduce the risk of novel vector-borne diseases being transmitted to the European continent from overseas. In this concern, it would be interesting to clarify whether SBV was already present on the African continent during the recent years without being noticed and identified. More studies on the biology and distribution of biting midges are needed in order to receive more detailed knowledge about these neglected species which might be potential carriers of not only animal-pathogenic but also human-pathogenic agents.

Limitations of this work derive from the usage of conservative non-parametric tests (Wilcoxon-two-sample-test and Kruskal-Wallis-test) used for statistical analysis of the collected data. These tests were chosen in order to compare whether influence variables as species, breed, housing conditions, federal states, treatment with repellents, exposure to wet- and woodland and time of mating might have any influence on the percentage of the within-herd-prevalence (%) as quantitative target variable. Manuscript I reports no statistically significant differences concerning the treatment with repellents or the exposure to wet- and woodland of the tested goat flocks whereas a statistically significant difference between housing conditions and percentage of within-herd-prevalence could be found. Manuscript II shows no significant difference concerning the median within-herd-prevalences of different sheep breeds although it was obvious that hair sheep breeds seemed to be less affected than other sheep breeds. A significant difference concerning the median within-herd-prevalence of sheep and goats was reported in manuscript II. Both statistical analyses were based on one value (median within-herd-prevalence of the flocks). The statistical power could have been improved by taking the number of investigated animals per flock into account (i.e. in total

1065 observations in manuscript I and 2260 observations in manuscript II). This would have been possible by using a logistic regression model for grouped (i.e. flocks) binominally scaled observations. By using this model for statistical analyses we might have been able to prove more statistically significant correlations as for example between different sheep breeds. Furthermore this simple regression model should have been extended to a common multivariable model regarding the problem of confounding. This phenomenon could be observed in manuscript I which revealed a lower seroprevalence for NRW than for the other federal states. This result is, however, presumably due to the fact that most goat flocks sampled in NRW were dairy goat flocks kept permanently indoors or at least indoors overnight. This means that the lower seroprevalence in NRW is a spurious correlation and has probably nothing to do with the region but with the housing system of the tested flocks. Moreover a mixed model with a random effect for herd should have been used to account for the large variation from herd to herd. Both studies showed a wide range of within-herd-prevalences of the flocks. This means that observations are strongly correlated with each other.

Hence, a model for logistic regression would have been helpful to improve the statistical power of this work and to clarify possible cause-effect relationships between influence variable and target variable in a more expressive way.

5. Summary

Carina Helmer

Investigations on the distribution and transmission of Schmallerberg virus in sheep and goat flocks

Schmallerberg virus (SBV) primarily entered Germany and almost all parts of Europe in 2011 as a new viral disease in ruminants. Clinical and pathomorphological findings are characterised by malformed and stillborn lambs and calves. These findings are caused by an intrauterine viral transmission from the mother animal to the embryo/foetus. SBV belongs to the family Bunyaviridae, genus Orthobunyavirus and is primarily transmitted by biting midges. Small ruminants were clinically affected to a much higher degree by SBV-infections than cattle resulting in partly massive economic losses for sheep and goat farmers. Malformed and stillborn lambs and goat kids as well as mother animals who died or had to be euthanized due to dystocia or obstructed labours were main reasons for these losses.

The aim of this study was to acquire data on the epidemiology and transmission patterns of this newly emerging viral disease. A SBV-specific questionnaire was raised in order to collect data regarding clinical symptoms of SBV-infections and operational data of the farms which might be helpful to understand the transmission and distribution of the disease.

Manuscript I outlines information about the distribution of the new virus within goat flocks in the thus far most affected federal states of Germany (Hesse, Lower Saxony, Mecklenburg Western-Pomerania, North Rhine-Westphalia, Saxony-Anhalt and Schleswig-Holstein) based on serological analyses. For this purpose 40 randomly selected goat flocks located in the six mentioned German federal states were asked for SBV-infections in their flocks and for operational data of their farms for the months January until May 2012. Concurrently, 1065 serum samples were obtained from female goats older than one year. These were analysed for antibodies against SBV by ELISA in order to detect the post exposure and the within-herd-

prevalence of SBV. Serological analysis revealed a median within-herd-prevalence of 36.7% (min-max: 0-93.3%). 95% of the flocks had antibodies against SBV. Only two flocks were seronegative. Hence, German goat flocks are distinctly lesser affected by SBV-infections than sheep flocks or cattle herds with within-herd-prevalences of 60% (sheep) or 80-90% (cattle), respectively.

Statistical analysis revealed a significant difference between goat flocks which are kept permanently indoors and goat flocks which are kept out on pasture day and night (P value: 0.0001). A possible explanation for these findings is the fact that permanently housed goats have a certain protection against *Culicoides spp.* which are the main vectors of SBV. Biting midges show a seasonal activity throughout the year with peaks from March until November. Furthermore biting midges are crepuscular or nocturnal. In contrast to most cattle herds in Germany, permanently housed goats are mainly kept on deep litter. Goat and sheep droppings are very arid and firm which makes it hard for larval stages of *Culicoides spp.* to immigrate as they develop aquatically. By contrast, cattle, especially intensive farmed dairy cattle and fattening bulls, are kept on slatted floors with cesspools below. Cattle slurry is very liquid and nutrient-rich. The storage of this liquid manure in huge cesspools provide perfect breeding and development conditions for larval stages of *Culicoides spp.*. Several studies describe that *Culicoides spp.* prefer cattle as blood meal hosts in comparison to small ruminants. These facts might also explain the differences concerning the within-herd-prevalences of cattle and small ruminants.

Manuscript II describes a serosurvey of SBV-infections of sheep and goat flocks in Lower Saxony, Germany. Therefore 81 randomly selected farmers of sheep and goat flocks (64 sheep and 17 goat flocks) were interviewed regarding SBV-infections in their flocks and asked for operating data of their farms for the months January until May 2012. Lower Saxony was located in the core region of the SBV epidemic in 2011/2012. Several sheep and goat farmers reported massive losses due to malformed newborns and dystocia. Serum samples were obtained from 2260 female sheep and goats older than one year. Serological analyses in order to detect antibodies against SBV were performed by ELISA. The calculated median within-herd-prevalence was 58.7% for sheep (min-max: 6.5-100%) and 43.8%

(min-max: 5.6-93.3%) for goats which is significantly lower (P value: 0.0058) than for sheep. Differences in the percentage of within-herd-prevalences of different sheep breeds could not be confirmed by statistical tests, but nevertheless it was evident that flocks of hair sheep breeds had lower within-herd-prevalences than wool sheep breeds.

Therefore manuscript II confirms the first results outlined in manuscript I, that goat flocks are less affected by SBV-infections than other domestic ruminant species. Possible explanations for these findings are the different housing and management systems as well as different host preferences of the virus carrying vectors. The species-specific odour of goats may also keep biting midges away from choosing goats as blood meal hosts.

Hence, both studies reveal that an infestation of German sheep and especially goat flocks is not nearly complete as many animals have not yet built any antibodies against SBV and are therefore not protected against SBV-infections. These occurrences may, however, result in the birth of malformed goat kids and sheep lambs in the following lambing seasons. This situation might contribute to establish an enzootic SBV situation in central and northern Europe. The only reliable prevention method would be vaccination but there is yet no licenced vaccine on the German market available. Currently several pharmaceutical companies are working on reliable vaccines and in the United Kingdom a provisional authorisation of an inactivated SBV vaccine was released for the summer period 2013. Further prevention methods to reduce the risk of SBV infections are strict and continuous treatments with repellents, housing of animals during mating and early pregnancy and rescheduling the mating period to the colder period of the year. However it must be admitted that all of these measures do not offer a 100% protection against SBV as most repellents only kill the insects after a blood meal. Thus, the virus might already been transmitted. *Culicoides spp.* might also be found inside stables, even during the coldest winter month although then in a much lower quantity.

Moreover, both studies outline that investigations and surveillance programs of *Culicoides spp.* were disregarded almost completely during the last decades. Information on mode of life and biology are rare as well as entomologists. Surveillance programs of

indigenous but also on exotic arthropod vectors should be encouraged and implemented after two vector borne epidemics (BTV and SBV) which ran over Europe in recent years. Furthermore, it would be advisable to control goods and animals not only for pathogenic agents but also for brought in insects and vermin as the increasing globalization and climate warming probably entail new unusual diseases for European conditions.

6. Zusammenfassung

Carina Helmer

Untersuchungen zu Vorkommen und Übertragungsmechanismen des Schmallenberg-Virus in Schaf- und Ziegenherden

Das Schmallenberg-Virus (SBV) trat 2011 als neue virale Erkrankung von Wiederkäuern erstmals in Deutschland und weiten Teilen Europas auf. Das klinische wie auch das pathologische Bild ist geprägt von missgebildeten und meist tot geborenen Lämmern und Kälbern, welches durch eine intrauterine Virusübertragung vom Muttertier auf den Embryo bzw. Fetus verursacht wird. Das SBV gehört zu den Bunyaviridae, Genus Orthobunyavirus und wird vornehmlich durch Gnitzen übertragen. Kleine Wiederkäuer waren klinisch sehr viel stärker von SBV-Infektionen betroffen als Rinder, was sich teilweise in sehr hohen wirtschaftlichen Verlusten der betroffenen Schaf- und Ziegenherden niederschlug. Missgebildete und meist tot geborene Lämmer, aber auch durch unüberwindbare Geburtsprobleme verendete oder euthanasierte Muttertiere, waren Hauptgründe für diese teils massiven Verluste.

Ziel dieser Arbeit war es, Daten über die Epidemiologie und Verbreitungswege dieser neu aufgetretenen Viruserkrankung zu erhalten. Durch einen SBV-spezifischen Fragebogen wurden Daten zum klinischen Bild der SBV-Infektion erhoben und Betriebsdaten erfragt, die mit einer Erkrankung der Herde in Zusammenhang stehen könnten.

Im ersten Teil der Arbeit (vgl. Manuskript I) konnten Erkenntnisse über die Verbreitung des neuen Virus innerhalb von Ziegenherden in den bis dato (2011/2012) am stärksten betroffenen deutschen Bundesländer (Hessen, Mecklenburg-Vorpommern, Niedersachsen, Nordrhein-Westfalen, Sachsen-Anhalt und Schleswig-Holstein) anhand von serologischen Untersuchungen auf Antikörper gegen SBV gewonnen werden. Dazu wurden von Januar bis Mai 2012 40 zufällig ausgesuchte Ziegenherden in den sechs besagten Bundesländern mit

einem SBV-spezifischen Fragebogen zu SBV-Infektionen in ihrem Bestand und Betriebsdaten befragt und insgesamt 1065 Serumbloodproben von weiblichen Ziegen im Alter von über einem Jahr gewonnen. Diese wurden anschließend serologisch mittels eines ELISA auf Antikörper gegen das SBV getestet, um herauszufinden, wie weit sich das neue Virus bereits ausgebreitet hat und wie hoch die Intra-Herden-Prävalenz innerhalb einer Herde ist. Die serologischen Untersuchungen ergaben eine mediane Intra-Herden-Prävalenz der 40 beprobten Ziegenherden von 36.7% (Min-Max: 0 - 93,3%). 95% der beprobten Ziegenherden wiesen Antikörper gegen das SBV auf. Nur zwei Betriebe waren serologisch negativ. Somit sind Ziegenherden in Deutschland deutlich weniger von SBV-Infektionen betroffen als Schaf- und auch Rinderherden, für welche die mediane Intra-Herden-Prävalenz mit 60% (Schaf) bzw. 80-90% (Rind) veranschlagt wird.

Mittels statistischer Analysen konnte ein signifikanter Unterschied zwischen Ziegenherden, die in ganzjähriger Stallhaltung gehalten werden und Ziegenherden, die ganzjährig draußen gehalten werden, festgestellt werden (P-Wert: 0,0001). Dieser Unterschied lässt sich dadurch erklären, dass die Ziegen in ganzjähriger Stallhaltung gegen die Überträger des SBV, vornehmlich *Culicoides spp.*, geschützt sind. Gnitzen zeigen in ihrer Aktivität saisonale Verteilungen mit Aktivitätsmaxima von März bis November. Des Weiteren sind die meisten Gnitzen dämmerungs- und nachtaktiv. Anders als die meisten Rinder in Deutschland werden Ziegen in Stallhaltung vornehmlich auf Tiefstreu gehalten. Schaf- und auch Ziegenstreu ist sehr fest und trocken und bietet Gnitzenlarven, welche sich aquatisch entwickeln, kaum Entfaltungsraum. Rinder hingegen, vor allem intensiv gehaltenen Milchkühe und Mastbullen, werden zum größten Teil auf Vollspaltenboden mit untergelagertem Güllekeller gehalten. Rindergülle ist sehr flüssig und nährstoffreich. Die Lagerung der flüssigen Gülle bietet Gnitzenlarven somit optimale Fortpflanzungs- und Entwicklungsbedingungen. Auch ist in diversen Studien beschrieben, dass *Culicoides spp.* Rinder als Blutmahlzeitenquelle kleinen Wiederkäuern gegenüber bevorzugen, was die Unterschiede in der Intra-Herden-Prävalenz erklären könnte.

Im zweiten Teil der Arbeit (vgl. Manuskript II) wurden von Januar bis Mai 2012 81 (64 Schaf- und 17 Ziegenherden) zufällig ausgewählte Schaf- und Ziegenherden in

Niedersachsen befragt und beprobt. Niedersachsen lag in der 2011/2012 erstmals auftretenden SBV Epidemie im Zentrum des Geschehens. Viele Schaf- und auch Ziegenhalter meldeten teilweise horrende Verluste auf Grund von missgebildeten Lämmern und unüberwindbaren Geburtshindernissen. Von insgesamt 2260 weiblichen Schafen und Ziegen im Alter von über einem Jahr wurden Serumblutproben entnommen und mittels ELISA auf Antikörper gegen das SBV untersucht. Die mediane Intra-Herden-Prävalenz lag für Schafe bei 58,7% (Min-Max: 6,5 – 100%) und für Ziegen signifikant niedriger (P-Wert: 0,0058) bei 43,8% (Min-Max: 5,6 – 93,3%). Unterschiede in der Intra-Herden-Prävalenz bezüglich einzelner Schafrassen konnten statistisch nicht gesichert werden. Es war jedoch auffällig, dass Herden von Haarschafen tendenziell geringere Intra-Herden-Prävalenzen aufwiesen als Wollschafressen.

Auch die zweite Studie kommt zu dem Schluss, dass Ziegenherden deutlich weniger von SBV Infektionen betroffen sind als andere domestizierte Wiederkäuer. Erklärungen für diese Ergebnisse liegen möglicherweise in unterschiedlichen Haltungsbedingungen und unterschiedlichen Wirtspräferenzen der Vektoren des Virus zu Grunde. Auch der speziesspezifische Geruch von Ziegen könnte Gnitzen vom Stechen abhalten und dadurch eine wichtige Rolle spielen.

Diese Untersuchungen verdeutlichen somit, dass sowohl Schaf- als auch Ziegenherden in Deutschland längst nicht vollständig mit dem SBV durchseucht sind und daher das Risiko von erneuten SBV-Infektionen mit möglicherweise hohen Lämmerverlusten in den nächsten Ablammperioden bestehen bleibt. Diese Umstände könnten dazu beitragen, dass SBV-Infektionen in Zentral- und Nordeuropa in Zukunft enzootisch auftreten. Zuverlässigen Schutz gegen erneute Infektionen würde zurzeit nur eine Impfung erzielen. In Deutschland ist derzeit aber noch kein zugelassener Impfstoff verfügbar. Diverse Pharmaunternehmen arbeiten unter Hochdruck an einer zuverlässigen Vakzine. In Großbritannien ist für die Sommermonate 2013 vorläufig ein Impfstoff zum Schutz gegen das SBV zugelassen worden. Weitere Präventionsmaßnahmen, um das Risiko von SBV-Infektionen einzudämmen, sind strikte und kontinuierliche Behandlungen mit Repellents, Stallhaltung während der Deckzeit und der ersten Trächtigkeitsmonate und eine Verlegung der Deckzeit in die kälteren Monate

des Jahres. Allerdings bieten alle diese Maßnahmen keinen vollständigen Schutz gegen das SBV, da kommerziell erhältliche Repellents die stechenden Insekten erst nach einer stattgefundenen Blutmahlzeit töten und das Virus somit schon übertragen worden sein könnte. Sogar in den kältesten Wintermonaten können noch Gnitzen in Stallgebäuden gefunden werden, wenn auch in deutlich reduzierter Anzahl.

Des Weiteren wird in beiden Studien deutlich, dass die Erforschung und Überwachung von *Culicoides ssp.* in den letzten Jahrzehnten völlig vernachlässigt worden ist. Es fehlt an Informationen zur Lebensweise und Biologie der Insekten und auch an Entomologen. Überwachungsprogramme einheimischer aber auch exotischer Insekten sollten nach zwei vektorübertragenen Epidemien, die uns in den letzten Jahren mit BTV und SBV ereilt haben, angeregt und in die Tat umgesetzt werden. Außerdem wäre es sehr sinnvoll, aus Übersee angelieferte Waren und Tiere nicht nur auf pathogene Keime, sondern auch auf mitgebrachte Insekten und Schädlinge zu kontrollieren, da die zunehmende Globalisierung und Klimaerwärmung sicherlich weitere für europäische Verhältnisse untypische Erkrankungen mit sich bringen wird.

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