Rev. Sci. Tech. Off. Int. Epiz., 2015, **34** (2), 363-373

Schmallenberg virus infection

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Summary
Since Schmallenberg virus, an orthobunyavirus of the Simbu serogroup, was identified near the German–Dutch border for the first time in late 2011 it has spread extremely quickly and caused a large epidemic in European livestock. The virus, which is transmitted by *Culicoides* biting midges, infects domestic and wild ruminants. Adult animals show only mild clinical symptoms or none at all, whereas an infection during a critical period of gestation can lead to abortion, stillbirth or the birth of severely malformed offspring. The impact of the disease is usually greater in sheep than in cattle. Vaccination could be an important aspect of disease control.

Keywords
Bunyaviridae – *Culicoides* midge – Europe – Orthobunyavirus – Ruminant – Teratogenic.

Introduction

Starting in late summer and autumn 2011, an unidentified disease in dairy cattle was reported by farmers and veterinarians in north-western Germany and the Netherlands. Clinical symptoms included a sudden decrease in milk production, fever and diarrhoea lasting for a few days (1, 2). A hitherto unknown orthobunyavirus was subsequently identified by metagenomic analyses in samples from acutely diseased cows on a farm near the city of Schmallenberg, Germany. In addition, using identical samples, the virus was isolated in insect and baby hamster kidney cells and subsequently named Schmallenberg virus (SBV) (1).

The virus predominantly infects domestic and wild ruminants. Thus, the viral genome and specific antibodies have been detected in cattle, sheep, goats, bison, moose, alpacas, buffalos, fallow deer, roe deer and red deer (3). Antibodies were also found in a dog in Sweden (4). Type I interferon receptor knock-out mice are susceptible to an SBV infection (5), but there is no evidence that humans can be infected (6, 7).

Aetiological agent

Schmallenberg virus is a member of the family *Bunyaviridae*, which is divided into the five genera *Orthobunyavirus*, *Hantavirus*, *Nairovirus*, *Phlebovirus* and *Tospovirus*. The genus *Orthobunyavirus*, to which SBV belongs, contains more than 170 viruses of differing medical and veterinary relevance (8) and is divided into 18 serogroups. Phylogenetic analyses suggested that SBV is a member of the Simbu serogroup, together with Akabane virus (AKAV), Aino virus (AINOV), Simbu virus, Douglas virus, Shamonda virus and Sathuperi virus, for example (9, 10).

Schmallenberg virions are spherical, enveloped particles approximately 80–120 nm in diameter (11). As in typical orthobunyaviruses, the negative-stranded tripartite RNA genome, which comprises a large (L), a medium (M) and a small (S) segment, encodes for six proteins. The orthobunyaviral L-segment encodes the RNA-dependent RNA polymerase, the M-segment encodes the viral glycoproteins Gn and Gc, as well as a non-structural protein, and the S-segment encodes the nucleocapsid (N) protein together with a small non-structural protein in an alternative overlapping reading frame (12, 13).

As described for related viruses such as AKAV (14), the M segment of SBV is the most variable of the three segments (15, 16, 17). A region of high sequence variability was found in field samples within the glycoprotein Gc (15, 16), and after several cell culture passages of cell culture-grown virus without immune selective pressure (16). This hypervariable region is independent of the host species or area from which the samples were derived (15); however, its relevance is unknown and needs further investigation.

As in other segmented viruses, genetic reassortment occurs between different bunyaviruses (14, 18, 19) but studies of the Simbu serogroup are very limited. It has been suggested that SBV is a reassortant between the M-segment of Sathuperi virus and the S- and L-segments of Shamonda virus (10), but in another report it was concluded that SBV is an ancestor of Shamonda virus, which is a reassortant containing the S- and L-segments of SBV and the M-segment from an unclassified virus. The latter assumption was supported by serological cross-reactivity (9).

Epidemiology

Serum samples taken during spring 2010 and in spring and summer 2011 from ruminants in Germany, the Netherlands, Belgium and France were all seronegative for SBV, suggesting the absence of the virus from central Europe before summer 2011 (20, 21, 22, 23). However, since its first detection in early autumn 2011 near the German–Dutch border, SBV has spread extremely quickly over large parts of the European continent (3, 24). After the first vector season (2011), a very high seroprevalence from approximately 70% to nearly 100% was observed in north-western Germany, Belgium and the Netherlands (11, 20, 25), which was initially the most affected area. In general, the percentage seropositivity was higher in cattle than in small ruminants (11).

In the second transmission period (2012), SBV was still circulating in the primary affected area, but at a low level. The seroprevalence in adult Belgian cattle remained high but was only about 20% in calves between 6 and 12 months of age (26). The virus also spread from the core region of the 2011 epidemic into previously uninfected or less-affected regions; for example, eastern European countries such as Poland (27), and southern Europe as far as Spain (28) and Greece (29). Abortions caused by SBV were also reported from Turkish regions bordering European Union countries (30).

Schmallenberg virus has now been detected in northern European countries: Denmark (31), Sweden (32), Norway (33, 34) and Finland (35). The virus has even spread above the latitude of 60°N, which exceeds the distribution of bluetongue virus serotype 8, an orbivirus transmitted by the same insect vectors as SBV, during the 2006 to 2009 bluetongue epidemic (36). Cases of SBV infection, confirmed using real-time polymerase chain reaction (PCR) or antibody detection, were also reported among domestic and sympatric wild ruminants grazing alpine meadows at an altitude of more than 2,000 m in the eastern Pyrenees in Spain (28). Spreading from continental Europe, SBV reached southern England, possibly by infected midges blown across the English Channel (37), and from there spread across the British Isles to Scotland (38, 39) and Ireland (40).

As in the German–Dutch–Belgian border region in 2011, a very high seroprevalence was observed in the most-affected area after the 2012 vector season. Based on retrospective detection of antibodies or genome in archived samples, SBV was found to have reached Austria most probably in July–August 2012, the seroprevalence in Austrian cattle exceeding 98% in October of that year. The seroprevalence in small ruminants, however, was lower and differed regionally (41). An equally rapid increase of the between-herd seroprevalence in cattle was found in Switzerland: seropositivity in bulk milk samples from Swiss dairy cattle was barely 20% in July 2012, but by December that year it had increased to 99.5% (42).

Despite high seroprevalence in central European livestock, SBV was still circulating in the 2012 and 2013 vector season, but at a low level (43). As an example, seven instances of viral genome detection were reported to the German Animal Disease Reporting System (TSN) between 1 January and 24 March, 2014 (44).

Modes of transmission

Members of the Simbu serogroup to which SBV belongs are transmitted by *Culicoides* midges and/or mosquitoes (45, 46). Following the discovery of the introduction of SBV to European countries at the end of 2011, SBV RNA was rapidly detected in archived samples of field-caught *Culicoides* (47, 48, 49, 50, 51). The following *Culicoides* species were implicated in transmission of SBV: *C. obsoletus* sensu stricto, *C. scoticus*, *C. chiopterus* and *C. dewulfi*. A recent study has implicated *C. nubeculosus* for the first time as a potential field vector of SBV in France (52).

Testing of individual *Culicoides* specimens in the Netherlands showed that the prevalence of SBV infection was five to ten times higher than bluetongue virus infection in *Culicoides* species in Europe between 2002 and 2008 (50). Vector biology was positively influenced by climatological circumstances in 2011, with a prolonged vector season (several weeks as the result of higher than normal temperatures), higher survival rate and increased vector abundance (rain

in summer, higher than normal temperatures in autumn). In a Dutch field study investigating the presence of SBV in mosquitoes overwintering at 11 ruminant farms in the Netherlands, where between November 2011 and January 2012 SBV circulation had been proven based on the presence of SBV RNA in the brains of malformed newborns, no evidence was found for the presence of SBV in hibernating mosquitoes (*Culex*, *Anopheles*, *Culiseta* spp.) collected from January to March 2012 (53). It was therefore suggested that mosquitoes do not play an important role, if any at all, in the persistence of SBV during the winter months in north-western Europe. Recently, oral competence studies for SBV in two widespread mosquito species (*Aedes albopictus*, *Culex pipiens*) in Spain (52) demonstrated that neither species was able to replicate SBV to transmissible levels, and it was concluded that they appear unlikely to play a major role in transmission.

Experimental infection studies with laboratory-reared lines of *Culicoides* at the Pirbright Institute (United Kingdom [UK]) have provided evidence of successful dissemination of SBV in *C. sonorensis* (54). The results for *C. nubeculosus* were more equivocal, and the probability of developing a transmissible infection was significantly lower than in *C. sonorensis*. The low rate of SBV infection in laboratory-reared *C. nubeculosus* was confirmed in recent experimental studies in Spain (52).

No SBV could be detected in nulliparous *Culicoides* midges caught in Belgium in May 2012 (55), thus providing an indication that transovarial transmission is not likely to occur. Nevertheless, this should be further investigated as it was recently reported that SBV RNA was detected in midges considered as nulliparous based on visual inspection in Poland in 2012 (56).

Renewed but short-lived circulation of SBV in parous midges of the subgenus *Avaritia* was observed in Belgium in August 2012, but no more positive pools were found from September 2012 onwards (55). In the Netherlands, only two of 42 pools of midges of the Obsoletus complex caught in 2012 tested weakly positive, indicating a relatively low viral load (43). At the level of an individual midge, the proportion of SBV-infected *Culicoides* of the Obsoletus complex caught in the same area and in a comparable period of the year was significantly lower in 2012 (0.1%; 1 of 1,050 tested) than in 2011 (0.56%; 13 of 2,300 tested). It was assumed that the level of SBV circulation was lower in this area in 2012 because only a small fraction of hosts was left susceptible to infection after the massive epidemic in 2011.

Oronasal inoculation of calves with culture-grown SBV did not lead to infection (57) and in-contact cattle and sheep remained seronegative in several animal trials (57, 58, 59), therefore direct horizontal transmission of SBV is highly unlikely. In contrast, vertical transmission of the virus from the dam to the fetus by transplacental infection does occur; the rate, however, appears low in cattle (60, 61).

In addition, SBV genome has been detected in bull semen (62, 63, 64), and it was demonstrated by subcutaneous inoculation of calves (65) and interferon receptor-deficient mice (63) that a few of the tested PCR-positive semen samples contained infectious virus. Whether an infection can be induced by insemination of cows with SBV-containing semen and whether this has epidemiological relevance remain to be evaluated.

Clinical signs

Infected adult ruminants may show no clinical signs or only nonspecific mild signs such as fever, diarrhoea or reduced milk production for a few days (1, 58). As described for viruses related to SBV (66, 67), the viraemic period is short lived (maximum six days) (1, 58) but viral genome is detectable for an extended period in the lymphoreticular system, particularly in the mesenteric lymph nodes (57, 58, 59). Much more important than the effect on adult ruminants is the induction of severe congenital malformations, premature birth or stillbirth, or the birth of mummified fetuses, when the dam is infected during a critical phase of gestation. For AKAV, which is closely related to SBV, the critical period in cattle is between the third and sixth month of gestation (68, 69, 70) and in sheep between days 30 and 50 (71, 72, 73). In all probability, this critical period is similar for SBV (60). However, not every infection of the dam with SBV or other Simbu serogroup viruses during that timeframe results in an abnormal course of gestation or leads to fetal antibody response (60, 61, 74). The birth of one malformed and one healthy lamb/kid within the same litter has been reported (75, 76). Calves or lambs congenitally infected with SBV may show varying degrees of deformation in the central nervous system (Figs 1 & 2) and/or skeletal muscles (Fig. 3), which is described as arthrogryposis-hydranencephaly syndrome. The most common malformations comprise mild to severe hypoplasia of the central nervous system (Figs 1 & 2), narrow spinal cords, poliomyelitis, porencephaly, arthrogryposis (Fig. 3), torticollis, kyphosis, lordosis, scoliosis, ankylosis and brachygnathia (76, 77, 78, 79, 80).

Diagnosis

Direct virus detection is based primarily on detection of the SBV genome in real-time quantitative reverse-transcription PCR assays (RT-qPCR) targeting either the L-, M- or S-segment (1, 81, 82). Of those, the S-segment-based assay has been shown to be the most suitable system for sensitive

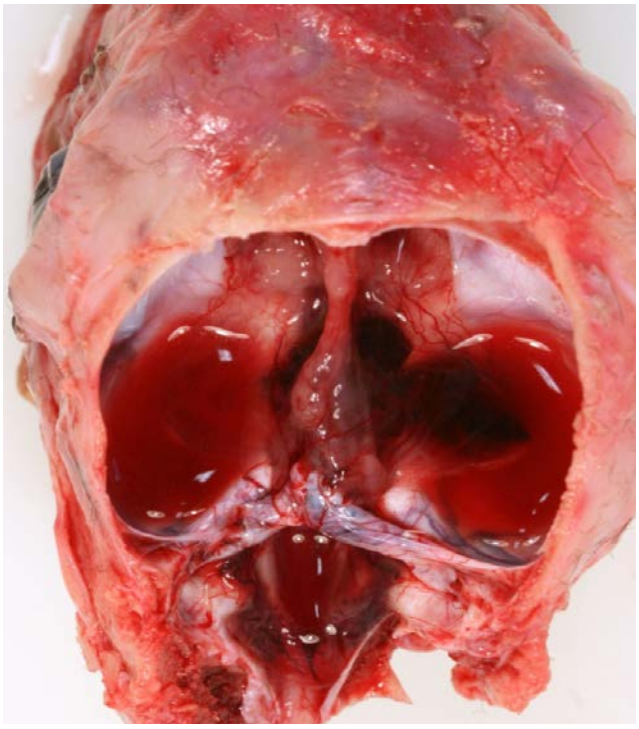

Fig. 1
Hydranencephaly and hypoplasia of the cerebellum caused by fetal infection with Schmallenberg virus

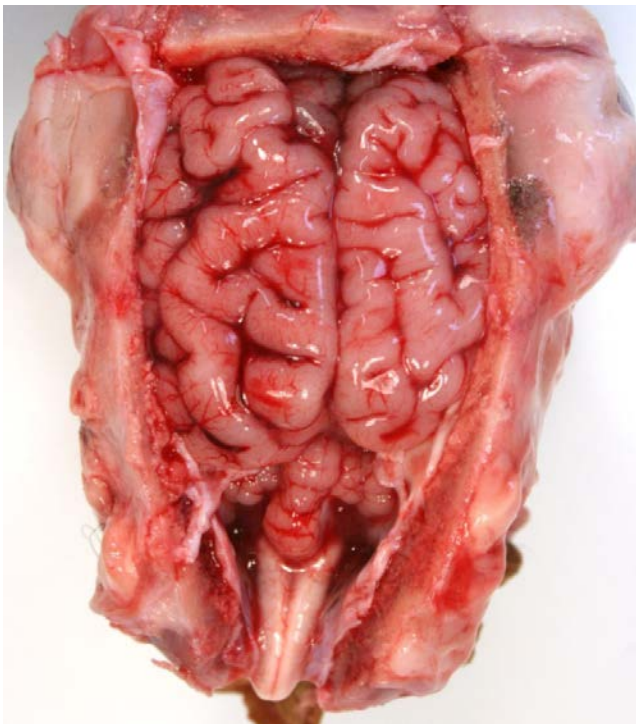

Fig. 2
Schmallenberg virus-induced hypoplasia of the cerebellum

Fig. 3
Schmallenberg virus-associated arthrogryposis in a lamb

and specific detection of SBV genome (81). In addition, for the detection of all members of the Simbu serogroup, a pan-Simbu RT-qPCR system with the possibility of subsequent species classification via sequencing has been established (81). Further, SBV can be isolated on insect cell lines (e.g. larval cells of *Culicoides variipennis*), baby hamster kidney cells or African green monkey (Vero) cells (1, 58).

However, because of the short viraemia lasting only a few days in acutely infected animals (1, 57, 58), detection of specific antibodies is much more promising than RT-qPCR or virus isolation. Test systems include micro-neutralisation, indirect immunofluorescence tests and commercially available enzyme-linked immunosorbent assays (ELISAs) (57, 83, 84, 85). Of those, the neutralisation test was found to be slightly more sensitive than the applied ELISAs in a limited ring trial among European laboratories (86).

The preferred sample material for detecting acute infections in adult ruminants and for antibody detection is serum. Suitable sample materials for RT-qPCR of aborted fetuses or malformed newborns include brain samples, placenta, meconium and hair swabs (82, 87, 88). However, viral genomes cannot be detected in all lambs or calves suspected of SBV infection (88, 89), which has also been described for SBV-related viruses such as AKAV (90). In addition to RT-qPCR, the detection of antibodies in fetal heart blood samples or in serum taken before colostrum intake is a valuable tool for confirmation of congenital SBV infection (60, 88, 89).

Impact

In general, the impact of SBV infection on animal welfare and animal production appears limited but is greater among sheep than among cattle (24). Recently, the between-herd

impact of SBV was calculated as the number of herds with one or more malformed virus-positive fetuses in relation to the number of holdings in the respective region. In the countries affected by SBV between 2011 and 2013, the 75th percentile of the case intensity was below 1% for cattle (maximum 4%) and 3% for sheep herds (maximum 7%) (36).

Based on the evaluation of questionnaires sent to large-animal veterinary practitioners in Belgium, the mean cost of individual symptomatic treatment was estimated to be between €40 and €200 in cases of apparent recovery and between €50 and €80 in cases with a fatal outcome (91). In adult cattle the most frequently observed symptoms were reduced milk production, diarrhoea, fever, abortion, dystocia and a few cases of mastitis; in adult sheep and goats almost only abortion and dystocia were reported (91). In Dutch dairy herds, the average daily milk yield per cow in 2011 was slightly lower in herds with SBV cases than in control herds (defined as herds without malformations in newborns or anomalies in reproductive performance but with seropositive animals) (61). Much more important than the very low effect on adult animals is the occasional induction of abortion, stillbirth and/or fetal malformation. Lambing data on French sheep flocks affected by SBV indicate that on average about 85% of the ewes gave birth normally. However, the clinical impact varied greatly between the flocks and most probably correlates with the percentage of ewes pregnant within the susceptible phase of gestation at the time of exposure to SBV. In approximately 12% of the SBV-affected herds the course of pregnancy was normal in more than 95% of the sheep, whereas in about 10% of the monitored farms fewer than half the ewes gave birth normally (92). On British sheep farms with RT-qPCR-confirmed SBV cases, on average 10.4% of all lambs that were born died within the first week after birth, and 8–16% of the farms with confirmed or suspected SBV infections had a lamb mortality of more than 40% (93). In contrast to sheep, the percentage of vertical transmission to their fetuses from cows infected during the critical phase of gestation and a subsequent abnormal course of pregnancy appears to be low. The number of stillborn and/or malformed calves born in Dutch dairy cattle herds was combined with calving data on the respective farms and showed that only approximately 0.5% of the calves born between February and September 2012 were infected with SBV (61). The relatively low number of aborted and/or malformed calves may also be applicable at farm level. In two closely monitored German cattle holdings, a very small proportion of calves whose mothers were naturally infected with SBV during the first five months of pregnancy were born dead, malformed, with detectable viral genome in meconium swabs or were seropositive before colostrum intake (60).

Control measures

As SBV is transmitted by *Culicoides* biting midges, the use of repellents or insecticides could be considered as a means of reducing the risk of exposure of susceptible animals to potentially infected vectors. However, a case-control study in Germany in 2012 provided no evidence for protective effects of such treatments (11). Another possibility for prevention of transplacental transmission to the fetus may be an intelligent breeding system where, for example, the number of cows and ewes in the susceptible stage of gestation could be minimised during the season of the highest activity of insect vectors responsible for virus transmission. Furthermore, grazing management could be adapted such that young stock is kept outside and is therefore more likely to be exposed to the vector; immunity against SBV acquired before heifers conceive for the first time might prevent fetal infections. Longitudinal whole-herd serological monitoring using a virus neutralisation test indicated that 80% of adult dairy cows still had measurable anti-SBV antibodies at least 24 months after the estimated introduction of virus into the herd. This means that at least two years after natural infection, animals were probably protected against re-infection (94).

Vaccination is a much more reliable method for acquiring immunity against the virus. It is known that inactivated vaccines against viruses closely related to SBV are able to prevent disease (95), making vaccination an adequate instrument for disease control. In Japan, for example, an inactivated, multivalent vaccine against AKAV, AINOV and the similarly teratogenic reovirus Chuzan virus is available to prevent infectious reproductive disorders in ruminants. However, this vaccine, although against viruses related to SBV, was not able to prevent an experimental SBV infection (96). In contrast to that heterologous vaccine, newly developed, inactivated, SBV-specific candidate vaccines have effectively protected cattle and sheep against an SBV infection (97). In May 2013, provisional marketing authorisation was granted for an inactivated vaccine against SBV for the UK market (98). Maternally derived antibodies, which may block the production of serum antibodies by young vaccinated animals, are detectable for a period of approximately 180 days (range 120–240) in calves (99).

Acknowledgement

Figures 1, 2 and 3 were kindly provided by M. Peters (Staatliches Veterinäruntersuchungsamt Arnsberg, Germany).

Infection due au virus Schmallenberg

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Résumé
Depuis la détection du virus Schmallenberg (un orthobunyavirus du sérogroupe Simbu) identifié pour la première fois en Allemagne, près de la frontière avec les Pays-Bas, à la fin de l'année 2011, ce virus s'est propagé à une grande vitesse, provoquant une pandémie majeure qui a affecté tout le bétail européen. Le virus, transmis par les moucherons du genre *Culicoides,* infecte les ruminants domestiques et sauvages. Les animaux adultes infectés ne présentent que peu ou pas de signes cliniques mais lorsque l'infection survient au cours d'un stade critique de la gestation, il peut s'ensuivre un avortement, la naissance de morts-nés ou de graves malformations congénitales chez la progéniture. L'impact de la maladie est généralement plus grave chez les ovins que chez les bovins. La vaccination pourrait jouer un rôle important dans la lutte contre cette maladie.

Mots-clés
Bunyaviridae – *Culicoides* – Europe – Moucheron – Orthobunyavirus – Ruminant – Teratogène.

Infección por el virus de Schmallenberg

K. Wernike, A. Elbers & M. Beer

Resumen
Desde que a finales de 2011 fue caracterizado cerca de la frontera entre Alemania y los Países Bajos, el virus de Schmallenberg, un orthobunyavirus del serogrupo Simbu, se ha extendido con suma rapidez y ha causado una gran epidemia en el ganado europeo. El virus, transmitido por el jején *Culicoides*, infecta a rumiantes domésticos y salvajes. Mientras que el animal adulto solo presenta signos clínicos leves, o en ocasiones ninguno, la infección en una fase crucial de la gestación puede causar aborto, mortinatalidad o graves malformaciones de la progenie. Las repercusiones de la enfermedad suelen ser más graves en el ganado ovino que en el vacuno. Un importante ingrediente de la lucha contra ella podría ser la vacunación.

Palabras clave
Bunyaviridae – Europa – Jején *Culicoides* – Orthobunyavirus – Rumiante – Teratógeno.

References

1. Hoffmann B., Scheuch M., Höper 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. *Emerg. Infect. Dis.*, **18** (3), 469–472.

2. Muskens J., Smolenaars A.J., van der Poel W.H., Mars M.H., van Wuijckhuise L., Holzhauer M., van Weering H. & Kock P. (2012). – Diarrhea and loss of production on Dutch dairy farms caused by the Schmallenberg virus. *Tijdschr. Diergeneeskd.*, **137** (2), 112–115.

3. European Food Safety Authority (EFSA) (2013). – 'Schmallenberg' virus: analysis of the epidemiological data (May 2013). EFSA Supporting Publications 2013. EN-3429. Available at: www.efsa.europa.eu/de/supporting/doc/429e.pdf (accessed on 15 July 2013).

4. Wensman J.J., Blomqvist G., Hjort M. & Holst B.S. (2013). – Presence of antibodies to Schmallenberg virus in a dog in Sweden. *J. Clin. Microbiol.*, **51** (8), 2802–2803.

5. Wernike K., Breithaupt A., Keller M., Hoffmann B., Beer M. & Eschbaumer M. (2012). – Schmallenberg virus infection of adult type I interferon receptor knock-out mice. *PLoS ONE*, **7** (7), e40380.

6. Ducomble T., Wilking H., Stark K., Takla A., Askar M., Schaade L., Nitsche A. & Kurth A. (2012). – Lack of evidence for Schmallenberg virus infection in highly exposed persons, Germany, 2012. *Emerg. Infect. Dis.*, **18** (8), 1333–1335.

7. 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. *Emerg. Infect. Dis.*, **18** (11), 1746–1754.

8. Plyusnin A., Beaty B.J., Elliott R.M., Goldbach R., Kormelink R., Lundkvist A., Schmaljohn C.S. & Tesh R.B. (2012). – *Bunyaviridae*. *In* Virus taxonomy: ninth report of the International Committee on Taxonomy of Viruses (A.M.Q. King, M.J. Adams, E.B. Carstens & E.J. Lefkowits, eds). Elsevier Academic Press, London, 725–741.

9. Goller K.V., Höper D., Schirrmeier H., Mettenleiter T.C. & Beer M. (2012). – Schmallenberg virus as possible ancestor of Shamonda virus. *Emerg. Infect. Dis.*, **18** (10), 1644–1646.

10. Yanase T., Kato T., Aizawa M., Shuto Y., Shirafuji H., Yamakawa M. & Tsuda T. (2012). – Genetic reassortment between Sathuperi and Shamonda viruses of the genus *Orthobunyavirus* in nature: implications for their genetic relationship to Schmallenberg virus. *Arch. Virol.*, **157** (8), 1611–1616.

11. Wernike K., Conraths F., Zanella G., Granzow H., Gache K., Schirrmeier H., Valas S., Staubach C., Marianneau P., Kraatz F., Höreth-Böntgen D., Reimann I., Zientara S. & Beer M. (2014). – Schmallenberg virus: two years of experiences. *Prev. Vet. Med.*, **116** (4), 423–434. doi:10.1016/j.prevetmed.2014.03.021.

12. Walter C.T. & Barr J.N. (2011). – Recent advances in the molecular and cellular biology of bunyaviruses. *J. Gen. Virol.*, **92** (11), 2467–2484.

13. Chowdhary R., Street C., Travassos da Rosa A., Nunes M.R., Tee K.K., Hutchison S.K., Vasconcelos P.F., Tesh R.B., Lipkin W.I. & Briese T. (2012). – Genetic characterization of the Wyeomyia group of orthobunyaviruses and their phylogenetic relationships. *J. Gen. Virol.*, **93** (5), 1023–1034.

14. Kobayashi T., Yanase T., Yamakawa M., Kato T., Yoshida K. & Tsuda T. (2007). – Genetic diversity and reassortments among Akabane virus field isolates. *Virus Res.*, **130** (1–2), 162–171.

15. Fischer M., Hoffmann B., Goller K.V., Höper D., Wernike K. & Beer M. (2013). – A mutation 'hot spot' in the Schmallenberg virus M segment. *J. Gen. Virol.*, **94** (6), 1161–1167.

16. Coupeau D., Claine F., Wiggers L., Kirschvink N. & Muylkens B. (2013). – In vivo and in vitro identification of a hypervariable region in Schmallenberg virus. *J. Gen. Virol.*, **94** (6), 1168–1174.

17. Hulst M., Kortekaas J., Hakze-van der Honing R., Vastenhouw S., Cornellissen J., van Maanen K., Bossers A., Harders F., Stockhofe N. & van der Poel W. (2013). – Genetic characterization of an atypical Schmallenberg virus isolated from the brain of a malformed lamb. *Virus Genes*, **47** (3), 505–514.

18. Briese T., Bird B., Kapoor V., Nichol S.T. & Lipkin W.I. (2006). – Batai and Ngari viruses: M segment reassortment and association with severe febrile disease outbreaks in East Africa. *J. Virol.*, **80** (11), 5627–5630.

19. Bowen M.D., Trappier S.G., Sanchez A.J., Meyer R.F., Goldsmith C.S., Zaki S.R., Dunster L.M., Peters C.J., Ksiazek T.G. & Nichol S.T. (2001). – A reassortant bunyavirus isolated from acute hemorrhagic fever cases in Kenya and Somalia. *Virology*, **291** (2), 185–190.

20. Garigliany M.M., Bayrou C., Kleijnen D., Cassart D. & Desmecht D. (2012). – Schmallenberg virus in domestic cattle, Belgium, 2012. *Emerg. Infect. Dis.*, **18** (9), 1512–1514.

21. Wernike K., Silaghi C., Nieder M., Pfeffer M. & Beer M. (2014). – Dynamics of Schmallenberg virus infection within a cattle herd in Germany, 2011. *Epidemiol. Infect.*, **142** (7), 1501–1504.

22. Zanella G., Raballand C., Durand B., Sailleau C., Pelzer S., Benoit F., Doceul V., Zientara S. & Bréard E. (2013). – Likely introduction date of Schmallenberg virus into France according to monthly serological surveys in cattle. *Transbound. Emerg. Dis.* E-pub.: 11 December 2013. doi:10.1111/tbed.12198.

23. Veldhuis A.M., van Schaik G., Vellema P., Elbers A.R., Bouwstra R., van der Heijden H.M. & Mars M.H. (2013). – Schmallenberg virus epidemic in the Netherlands: Spatiotemporal introduction in 2011 and seroprevalence in ruminants. *Prev. Vet. Med.*, **112** (1–2), 35–47. doi:10.1016/j.prevetmed.2013.06.010.

24. European Food Safety Authority (EFSA) (2012). – 'Schmallenberg' virus: analysis of the epidemiological data. EFSA Supporting Publications. EN-360. Available at: www.efsa.europa.eu/en/supporting/doc/360e.pdf (accessed on 15 July 2013).

25. Elbers A.R., Loeffen W.L., Quak S., de Boer-Luijtze E., van der Spek A.N., Bouwstra R., Maas R., Spierenburg M.A., de Kluijver E.P., van Schaik G. & van der Poel W.H. (2012). – Seroprevalence of Schmallenberg virus antibodies among dairy cattle, the Netherlands, winter 2011–2012. *Emerg. Infect. Dis.*, **18** (7), 1065–1071.

26. Meroc E., Poskin A., Van Loo H., Van Driessche E., Czaplicki G., Quinet C., Riocreux F., De Regge N., Caij B., van den Berg T., Hooyberghs J. & Van der Stede Y. (2013). – Follow-up of the Schmallenberg virus seroprevalence in Belgian cattle. *Transbound. Emerg. Dis*. E-pub.: 12 December 2013. doi:10.1111/tbed.12202.

27. Larska M., Krzysiak M., Smreczak M., Polak M.P. & Zmudzinski J.F. (2013). – First detection of Schmallenberg virus in elk (*Alces alces*) indicating infection of wildlife in Bialowieza National Park in Poland. *Vet. J.*, **198** (1), 279–281.

28. Fernández-Aguilar X., Pujols J., Velarde R., Rosell R., López-Olvera J.R., Marco I., Pumarola M., Segales J., Lavin S. & Cabezon O. (2014). – Schmallenberg virus circulation in high mountain ecosystem, Spain. *Emerg. Infect. Dis.*, **20** (6), 1062–1064.

29. Chaintoutis S.C., Kiossis E., Giadinis N.D., Brozos C.N., Sailleau C., Viarouge C., Bréard E., Papanastassopoulou M., Zientara S., Papadopoulos O. & Dovas C.I. (2014). – Evidence of Schmallenberg virus circulation in ruminants in Greece. *Trop. Anim. Hlth Prod.*, **46** (1), 251–255. doi:10.1007/s11250-013-0449-5.

30. Yilmaz H., Hoffmann B., Turan N., Cizmecigil U.Y., Richt J.A. & van der Poel W.H. (2014). – Detection and partial sequencing of Schmallenberg virus in cattle and sheep in Turkey. *Vector Borne Zoonotic Dis.*, **14** (3), 223–225. doi:10.1089/vbz.2013.1451.

31. Rasmussen L.D., Kirkeby C., Bodker R., Kristensen B., Rasmussen T.B., Belsham G.J. & Bøtner A. (2012). – Rapid spread of Schmallenberg virus-infected biting midges (*Culicoides* spp.) across Denmark in 2012. *Transbound. Emerg. Dis.*, **61** (1), 12–16. doi:10.1111/tbed.12189.

32. ProMED-mail (2012). – Schmallenberg virus - Europe (62): Sweden (Blekinge), positive serology. Archive Number: 20121005.1326266. Available at: www.promedmail.org (posted on 5 October 2012).

33. ProMED-mail (2012). – Schmallenberg virus – Europe (73): Norway, Sweden, update. Archive Number: 20121128.1428668. Available at: www.promedmail.org (posted on 28 November 2012).

34. Wisloff H., Nordvik B.S., Sviland S. & Tonnessen R. (2014). – The first documented clinical case of Schmallenberg virus in Norway: fetal malformations in a calf. *Vet. Rec.*, **174** (5), 120. doi:10.1136/vr.102149.

35. ProMED-mail (2013). – Schmallenberg virus - Europe (04): Finland confirmed, UK update. Archive Number: 20130115.1499846. Available at: www.promedmail.org (posted on 15 January 2013).

36. Afonso A., Abrahantes J.C., Conraths F., Veldhuis A., Elbers A., Roberts H., Van der Stede Y., Meroc E., Gache K. & Richardson J. (2014). – The Schmallenberg virus epidemic in Europe: 2011–2013. *Prev. Vet. Med.*, **116** (4), 391–403. doi:10.1016/j.prevetmed.2014.02.012.

37. Anon. (2012). – Schmallenberg virus detected in sheep in England. *Vet. Rec.*, **170** (4), 89.

38. Anon. (2013). – First case of Schmallenberg disease confirmed in Scotland. *Vet. Rec.*, **172** (17), 435.

39. Mason C., Stevenson H., Carty H., Hosie B., Caldow G. & Boyes G. (2013). – SBV in a dairy herd in Scotland. *Vet. Rec.*, **172** (15), 403.

40. Bradshaw B., Mooney J., Ross P.J., Furphy C., O'Donovan J., Sanchez C., Gomez-Parada M. & Toolan D. (2012). – Schmallenberg virus cases identified in Ireland. *Vet. Rec.*, **171** (21), 540–541.

41. Steinrigl A., Schiefer P., Schleicher C., Peinhopf W., Wodak E., Bago Z. & Schmoll F. (2014). – Rapid spread and association of Schmallenberg virus with ruminant abortions and foetal death in Austria in 2012/2013. *Prev. Vet. Med.*, **116** (4), 350–359. doi:10.1016/j.prevetmed.2014.03.006.

42. Balmer S., Vögtlin A., Thur B., Buchi M., Abril C., Houmard M., Danuser J. & Schwermer H. (2014). – Serosurveillance of Schmallenberg virus in Switzerland using bulk tank milk samples. *Prev. Vet. Med.*, **116** (4), 370–379. doi:10.1016/j.prevetmed.2014.03.026.

43. Elbers A.R., Meiswinkel R., van Weezep E., Kooi E.A. & van der Poel W.H. (2013). – Schmallenberg virus in *Culicoides* biting midges in the Netherlands in 2012. *Transbound. Emerg. Dis.*, E-pub.: 24 July 2013. doi:10.1111/tbed.12128.

44. Friedrich-Loeffler-Institut (2014). – Current information on Schmallenberg virus. Available at: www.fli.bund.de/en/startseite/current-news/animal-disease-situation/new-orthobunyavirus-detected-in-cattle-in-germany.html (accessed in June 2014.).

45. Elliott R.M. & Blakqori G. (2011). – Molecular biology of orthobunyaviruses. *In Bunyaviridae*: molecular and cellular biology (A. Plyusnin & R.M. Elliott, eds). Caister Academic Press, Norfolk, United Kingdom, 1–39.

46. Saeed M.F., Li L., Wang H., Weaver S.C. & Barrett A.D. (2001). – Phylogeny of the Simbu serogroup of the genus *Bunyavirus*. *J. Gen. Virol.*, **82** (9), 2173–2181.

47. De Regge N., Deblauwe I., De Deken R., Vantieghem P., Madder M., Geysen D., Smeets F., Losson B., van den Berg T. & Cay A.B. (2012). – Detection of Schmallenberg virus in different *Culicoides* spp. by real-time RT-PCR. *Transbound. Emerg. Dis.*, **59** (6), 471–475.

48. Rasmussen L.D., Kristensen B., Kirkeby C., Rasmussen T.B., Belsham G.J., Bodker R. & Bøtner A. (2012). – Culicoids as vectors of Schmallenberg virus. *Emerg. Infect. Dis.*, **18** (7), 1204–1206.

49. Goffredo M., Monaco F., Capelli G., Quaglia M., Federici V., Catalani M., Montarsi F., Polci A., Pinoni C., Calistri P. & Savini G. (2013). – Schmallenberg virus in Italy: a retrospective survey in *Culicoides* stored during the bluetongue Italian surveillance program. *Prev. Vet. Med.*, **111** (3–4), 230–236.

50. Elbers A.R., 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. *Emerg. Infect. Dis.*, **19** (1), 106–109.

51. Larska M., Polak M.P., Grochowska M., Lechowski L., Zwiazek J.S. & Zmudzinski J.F. (2013). – First report of Schmallenberg virus infection in cattle and midges in Poland. *Transbound. Emerg. Dis.*, **60** (2), 97–101.

52. Balenghien T., Pagès N., Goffredo M., Carpenter S., Augot D., Jacquier E., Talavera S., Monaco F., Depaquit J., Grillet C., Pujols J., Satta G., Kasbari M., Setier-Rio M.L., Izzo F., Alkan C., Delécolle J.C., Quaglia M., Charrel R., Polci A., Bréard E., Federici V., Cêtre-Sossah C. & Garros C. (2014). – The emergence of Schmallenberg virus across *Culicoides* communities and ecosystems in Europe. *Prev. Vet. Med.*, **116** (4), 360–369. doi:10.1016/j.prevetmed.2014.03.007.

53. Scholte E.J., Mars M.H., Braks M., Den Hartog W., Ibañez-Justicia A., Koopmans M., Koenraadt J.C., De Vries A. & Reusken C. (2013). – No evidence for the persistence of Schmallenberg virus in overwintering mosquitoes. *Med. Vet. Entomol.*, **28** (1), 110–115. doi:10.1111/mve.12010.

54. Veronesi E., Henstock M., Gubbins S., Batten C., Manley R., Barber J., Hoffmann B., Beer M., Attoui H., Mertens P.P. & Carpenter S. (2013). – Implicating *Culicoides* biting midges as vectors of Schmallenberg virus using semi-quantitative RT-PCR. *PLoS ONE*, **8** (3), e57747.

55. De Regge N., Madder M., Deblauwe I., Losson B., Fassotte C., Demeulemeester J., Smeets F., Tomme M. & Cay A.B. (2014). – Schmallenberg virus circulation in *Culicoides* in Belgium in 2012: field validation of a real time RT-PCR approach to assess virus replication and dissemination in midges. *PLoS ONE*, **9** (1), e87005.

56. Larska M., Lechowski L., Grochowska M. & Zmudzinski J.F. (2013). – Detection of the Schmallenberg virus in nulliparous *Culicoides obsoletus/scoticus* complex and *C. punctatus*: the possibility of transovarial virus transmission in the midge population and of a new vector. *Vet. Microbiol.*, **66** (3–4), 467–473.

57. Wernike K., Eschbaumer M., Schirrmeier H., Blohm U., Breithaupt A., Hoffmann B. & Beer M. (2013). – Oral exposure, reinfection and cellular immunity to Schmallenberg virus in cattle. *Vet. Microbiol.*, **165** (1–2), 155–159.

58. Wernike K., Hoffmann B., Bréard E., Bøtner A., Ponsart C., Zientara S., Lohse L., Pozzi N., Viarouge C., Sarradin P., Leroux-Barc C., Riou M., Laloy E., Breithaupt A. & Beer M. (2013). – Schmallenberg virus experimental infection of sheep. *Vet. Microbiol.*, **166** (3–4), 461–466.

59. Wernike K., Eschbaumer M., Breithaupt A., Hoffmann B. & Beer M. (2012). – Schmallenberg virus challenge models in cattle: infectious serum or culture-grown virus? *Vet. Res.*, **43** (1), 84–87.

60. Wernike K., Holsteg M., Schirrmeier H., Hoffmann B. & Beer M. (2014). – Natural infection of pregnant cows with Schmallenberg virus: a follow-up study. *PLoS ONE*, **9** (5), e98223.

61. Veldhuis A.M., Carp-van Dijken S., van Wuijckhuise L., Witteveen G. & van Schaik G. (2014). – Schmallenberg virus in Dutch dairy herds: potential risk factors for high within-herd seroprevalence and malformations in calves, and its impact on productivity. *Vet. Microbiol.*, **168** (2–4), 281–293.

62. Hoffmann B., Schulz C. & Beer M. (2013). – First detection of Schmallenberg virus RNA in bovine semen, Germany, 2012. *Vet. Microbiol.*, **167** (3–4), 289–295.

63. Ponsart C., Pozzi N., Bréard E., Catinot V., Viard G., Sailleau C., Viarouge C., Gouzil J., Beer M., Zientara S. & Vitour D. (2014). – Evidence of excretion of Schmallenberg virus in bull semen. *Vet. Res.*, **45** (1), 37–42.

64. Van der Poel W.H., Parlevliet J.M., Verstraten E.R., Kooi E.A., Hakze-van der Honing R. & Stockhofe N. (2014). – Schmallenberg virus detection in bovine semen after experimental infection of bulls. *Epidemiol. Infect.*, **142** (7), 1495–1500.

65. Schulz C., Wernike K., Beer M. & Hoffmann B. (2014). – Infectious Schmallenberg virus from bovine semen, Germany. *Emerg. Infect. Dis.*, **20** (2), 337–339.

66. St George T.D. & Kirkland P.D. (2004). – Diseases caused by Akabane and related Simbu-group viruses. *In* Infectious diseases of livestock (J.A.W. Coetzer & R.C. Tustin, eds), 2nd Ed. Oxford University Press, Oxford, 1029–1036.

67. Radostits O.M., Gay C.C., Hinchcliff K.W. & Constable P.D. (eds) (2007). – Congenital arthrogryposis and hydranencephaly; Akabane disease; Cache Valley virus disease. *In* Veterinary medicine: a textbook of the diseases of cattle, horses, sheep, pigs and goats, 10th Ed. Saunders Ltd, Philadelphia, 1207–1209.

68. Konno S., Moriwaki M. & Nakagawa M. (1982). – Akabane disease in cattle: congenital abnormalities caused by viral infection. Spontaneous disease. *Vet. Pathol.*, **19** (3), 246–266.

69. Kirkland P.D., Barry R.D., Harper P.A. & Zelski R.Z. (1988). – The development of Akabane virus-induced congenital abnormalities in cattle. *Vet. Rec.*, **122** (24), 582–586.

70. Kurogi H., Inaba Y., Takahashi E., Sato K. & Satoda K. (1977). – Congenital abnormalities in newborn calves after inoculation of pregnant cows with Akabane virus. *Infect. Immun.*, **17** (2), 338–343.

71. Hashiguchi Y., Nanba K. & Kumagai T. (1979). – Congenital abnormalities in newborn lambs following Akabane virus infection in pregnant ewes. *Natl Inst. Anim. Hlth Q. (Tokyo)*, **19** (1–2), 1–11.

72. Parsonson I.M., Della-Porta A.J. & Snowdon W.A. (1977). – Congenital abnormalities in newborn lambs after infection of pregnant sheep with Akabane virus. *Infect. Immun.*, **15** (1), 254–262.

73. Parsonson I.M., McPhee D.A., Della-Porta A.J., McClure S. & McCullagh P. (1988). – Transmission of Akabane virus from the ewe to the early fetus (32 to 53 days). *J. Comp. Pathol.*, **99** (2), 215–227.

74. Parsonson I.M., Della-Porta A.B., Snowdon W.A. & Murray M.D. (1975). – Letter: Congenital abnormalities in foetal lambs after inoculation of pregnant ewes with Akabane virus. *Aust. Vet. J.*, **51** (12), 585–586.

75. Anon. (2012). – Geographical spread of UK cases of SBV 'within expectations'. *Vet. Rec.*, **170** (9), 217.

76. 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. *Tijdschr. Diergeneeskd.*, **137** (2), 106–111.

77. Garigliany M.M., Hoffmann B., Dive M., Sartelet A., Bayrou C., Cassart D., Beer M. & Desmecht D. (2012). – Schmallenberg virus in calf born at term with porencephaly, Belgium. *Emerg. Infect. Dis.*, **18** (6), 1005–1006.

78. Peperkamp K., Dijkman R., van Maanen C., Vos J., Wouda W., Holzhauer M., van Wuijckhuise L., Junker K., Greijdanus S. & Roumen M. (2012). – Letter: Polioencephalo-myelitis in a calf due to infection with Schmallenberg virus. *Vet. Rec.*, **170** (22), 570.

79. Herder V., Wohlsein P., Peters M., Hansmann F. & Baumgartner W. (2012). – Salient lesions in domestic ruminants infected with the emerging so-called Schmallenberg virus in Germany. *Vet. Pathol.*, **49** (4), 588–591.

80. Anon. (2012). – Findings in cases of SBV infection in sheep in the south-east. *Vet. Rec.*, **170** (20), 510–513.

81. Fischer M., Schirrmeier H., Wernike K., Wegelt A., Beer M. & Hoffmann B. (2013). – Development of a pan-Simbu real-time reverse transcriptase PCR for the detection of Simbu serogroup viruses and comparison with SBV diagnostic PCR systems. *Virol. J.*, **10** (1), 327–335.

82. Bilk S., Schulze C., Fischer M., Beer M., Hlinak A. & Hoffmann B. (2012). – Organ distribution of Schmallenberg virus RNA in malformed newborns. *Vet. Microbiol.*, **159** (1–2), 236–238.

83. 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 nucleocapsid recombinant protein for detection of Schmallenberg virus antibodies. *PLoS ONE*, **8** (1), e53446.

84. Humphries D. & Burr P. (2012). – Schmallenberg virus milk antibody ELISA. *Vet. Rec.*, **171** (20), 511–512.

85. 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 Vet. Scand.*, **54** (1), 44–51.

86. Van der Poel W.H., Cay A.B., Zientara S., Steinbach F., Valarcher J.F., Bøtner A., Mars M.H., Hakze-van der Honing R., Schirrmeier H. & Beer M. (2014). – Limited interlaboratory comparison of Schmallenberg virus antibody detection in serum samples. *Vet. Rec.*, **174** (15), 380–383.

87. De Regge N., Van Loo H., Bertels G., Saulmont M., Vandenbussche F., van den Berg T. & Cay A.B. (2012). – Comparative analysis to determine the most appropriate tissue for Schmallenberg virus detection by RT-PCR in aborted lambs and calves. *In* Proc. Epizone 6th Annual Meeting, 12–14 June, Brighton, United Kingdom, p.13.

88. De Regge N., van den Berg T., Georges L. & Cay A.B. (2013). – Diagnosis of Schmallenberg virus infection in malformed lambs and calves and first indications for virus clearance in the fetus. *Vet. Microbiol.*, **162** (2–4), 595–600.

89. Van Maanen C., van der Heijden H., Wellenberg G.J., Witteveen G., Luttikholt S., Bouwstra R., Kooi B., Vellema P., Peperkamp K. & Mars J. (2012). – Schmallenberg virus antibodies in bovine and ovine fetuses. *Vet. Rec.*, **171** (12), 299–301.

90. Hartley W.J., De Saram W.G., Della-Porta A.J., Snowdon W.A. & Shepherd N.C. (1977). – Pathology of congenital bovine epizootic arthrogryposis and hydranencephaly and its relationship to Akabane virus. *Aust. Vet. J.*, **53** (7), 319–325.

91. Martinelle L., Dal Pozzo F., Gauthier B., Kirschvink N. & Saegerman C. (2014). – Field veterinary survey on clinical and economic impact of Schmallenberg virus in Belgium. *Transbound. Emerg. Dis.*, **61** (3), 285–288.

92. Dominguez M., Hendrikx P., Zientara S., Calavas D., Jay M., Touratier A., Languille J. & Fediaevsky A. (2012). – Preliminary estimate of Schmallenberg virus infection impact in sheep flocks: France. *Vet. Rec.*, **171** (17), 426–428.

93. Harris K.A., Eglin R.D., Hayward S., Milnes A., Davies I., Cook A.J. & Downs S.H. (2014). – The impact of Schmallenberg virus on British sheep farms during the 2011/2012 lambing season. *Vet. Rec.*, **175** (7), 172. doi:10.1136/vr.102295.

94. Elbers A.R., Stockhofe-Zurwieden N. & van der Poel W.H. (2014). – Schmallenberg virus antibody persistence in adult cattle after natural infection and decay of maternal antibodies in calves. *BMC Vet. Res.*, **10** (1), 103–106.

95. Kim Y.H., Kweon C.H., Tark D.S., Lim S.I., Yang D.K., Hyun B.H., Song J.Y., Hur W. & Park S.C. (2011). – Development of inactivated trivalent vaccine for the teratogenic Aino, Akabane and Chuzan viruses. *Biologicals*, **39** (3), 152–157.

96. Hechinger S., Wernike K. & Beer M. (2013). – Evaluating the protective efficacy of a trivalent vaccine containing Akabane virus, Aino virus and Chuzan virus against Schmallenberg virus infection. *Vet. Res.*, **44** (1), 114–119.

97. Wernike K., Nikolin V.M., Hechinger S., Hoffmann B. & Beer M. (2013). – Inactivated Schmallenberg virus prototype vaccines. *Vaccine*, **31** (35), 3558–3563.

98. Anon. (2013). – VMD authorises SBV vaccine for use in the UK. *Vet. Rec.*, **172** (21), 543.

99. Elbers A.R., Stockhofe N. & van der Poel W.H. (2014). – Schmallenberg virus antibodies in adult cows and maternal antibodies in calves. *Emerg. Infect. Dis.*, **20** (5), 901–902.