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Akabane, Aino and Schmallenberg virus — where do we stand and what do we know about the role of domestic ruminant hosts and *Culicoides* vectors in virus transmission and overwintering?

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Akabane, Aino and Schmallenberg virus belong to the Simbu serogroup of Orthobunyaviruses and depend on *Culicoides* vectors for their spread between ruminant hosts. Infections of adults are mostly asymptomatic or associated with only mild symptoms, while transplacental crossing of these viruses to the developing fetus can have important teratogenic effects. Research mainly focused on congenital malformations has established a correlation between the developmental stage at which a fetus is infected and the outcome of an Akabane virus infection. Available data suggest that a similar correlation also applies to Schmallenberg virus infections but is not yet entirely conclusive. Experimental and field data furthermore suggest that Akabane virus is more efficient in inducing congenital malformations than Aino and Schmallenberg virus, certainly in cattle.The mechanism by which these Simbu viruses cross-pass yearly periods of very low vector abundance in temperate climate zones remains undefined. Yearly wind-borne reintroductions of infected midges from tropical endemic regions with year-round vector activity have been proposed, just as overwintering in long-lived adult midges. Experimental and field data however indicate that a role of vertical virus transmission in the ruminant host currently cannot be excluded as an overwintering mechanism. More studies on *Culicoides* biology and specific groups of transplacentally infected newborn ruminants without gross malformations are needed to shed light on this matter.

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Introduction

Akabane (AKAV), Aino (AINOV) and Schmallenberg virus (SBV) are three viruses belonging to the Simbu serogroup of Orthobunyaviruses. All three are known to have caused outbreaks of congenital malformations in cattle, sheep and goats. After pregnant female adult ruminants get infected via the bite of an infected *Culicoides* in search of a blood meal, transplacental transfer of the virus to the developing fetus can occur. Subsequent virus replication in the fetus can then lead to congenital defects that are mostly only observed at birth. Several review papers are available that summarize field and experimental data showing that the clinical outcome and pathological lesions induced by a transplacental AKAV infection are strongly influenced by the stage of gestation at which the dam is infected, certainly in calves. Here we summarize data available for AINOV and SBV on this subject and evaluate whether similar conclusions can be made.

Furthermore, the epidemiology of these viruses is closely related to the presence of competent *Culicoides* vectors for their transmission and susceptible ruminant hosts that allow sufficient virus replication to provide an infectious blood meal to the insect vector. Although competent vectors sometimes remain present yearlong in tropical regions, they are often almost completely absent during unfavorable winter periods in temperate climate zones. Little evidence and literature data are available on the mechanisms used by AKAV, AINOV and SBV to cross-pass these periods of several months in the absence of *Culicoides* vectors. We summarize current working hypotheses and assessed whether sufficient experimental and field data are available to exclude the possibility that also vertical transplacental transmission in the ruminant host might be involved in overwintering and subsequent virus transmission of these viruses.

Virus biology

On the basis of the recently implemented changes by the International Committee on Taxonomy of Viruses [1], AKAV, AINOV and SBV are now considered exemplar viruses of the species *Akabane orthobunyavirus*, *Shuni orthobunyavirus* and *Sathuperi orthobunyavirus*, respectively, all three belonging to the genus *Orthobunyavirus*. Together with the genus *Herbevirus*, it forms the family *Peribunyaviridae*, part of the order *Bunyavirales*. Before this update,

AKAV, AINOV and SBV were grouped within the Simbu serogroup of *Orthobunyaviruses*, family *Bunyaviridae* [2].

Orthobunyaviruses are enveloped viruses consisting of a three segmented negative-stranded RNA genome covered by nucleocapsid proteins. The small (S) RNA segment encodes the nucleocapsid (N) protein and a non-structural (NSs) protein. The medium (M) RNA segment encodes a polyprotein precursor that is later cleaved into the non-structural (NSm) protein and the two envelop glycoproteins named Gn and Gc. These latter are known to be important for virus attachment, cell fusion, hemagglutination and induction of neutralizing antibodies by *Orthobunyaviruses*. The large (L) RNA segment encodes the RNA-dependent RNA polymerase [3].

For both AKAV and SBV, the S and L segments of different isolates obtained over time in concise geographical regions seem relatively genetically stable, while the M segment shows more variability [4–12]. A high genetic stability has also been documented for the S segment of AINOV [13]. Phylogenetic studies suggest that genetic reassortments occur among viruses of the Simbu serogroup [14–16].

Geographical spread and epizootics of congenital disease

AKAV was named after the village in Japan where the mosquitoes were collected from which the virus was first isolated in 1959 [17]. The presence of AKAV has since then been reported in tropical and temperate climate zones in Africa, Asia, Australia and the Middle East [18] and most data from outbreaks of clinical disease come from Australia, Japan, Israel and Korea. Although epizootics of congenital defects in cattle have been reported from the 1930s in Australia, the outbreaks of congenital malformation in Australia and Japan in the beginning of the 1970s really started the process of identification of AKAV as the etiological agent for the observed disease. AKAV is detected on an almost yearly basis in Japan, Australia, and Israel but epizootics of congenital malformations have a cyclic appearance [18,19•,20•,21]. These outbreaks are determined by the distribution of the vector and the level of population immunity against the virus [18]. Epizootic cycles are reported to occur every 3–5 years in Japan [19•], while 3 large outbreaks have been reported from Israel [20•]. In Australia, AKAV is considered to be endemic in a large region which coincides with the distribution range of the vector and which is characterized by a high level of population immunity. Outbreaks of congenital malformations occur when environmental conditions allow midges to disperse beyond their normal range where populations of susceptible livestock are present or when adverse climate conditions prevent the vectors from circulating within a part of their normal distribution range for some time, giving rise to naïve susceptible cattle. Also permanent or temporal movement of pregnant naïve animals into a vector area might provoke outbreaks of AKAV disease [22].

The first isolation of AINOV was made from mosquitoes collected in Japan in 1964 [23] and the virus appears to have a similar distribution as AKAV [13]. It is only since the large epizootic of congenital malformations in calves in Japan in 1995–1996 that its role as an etiological agent was confirmed [24]. Literature on this virus is limited compared to AKAV and SBV.

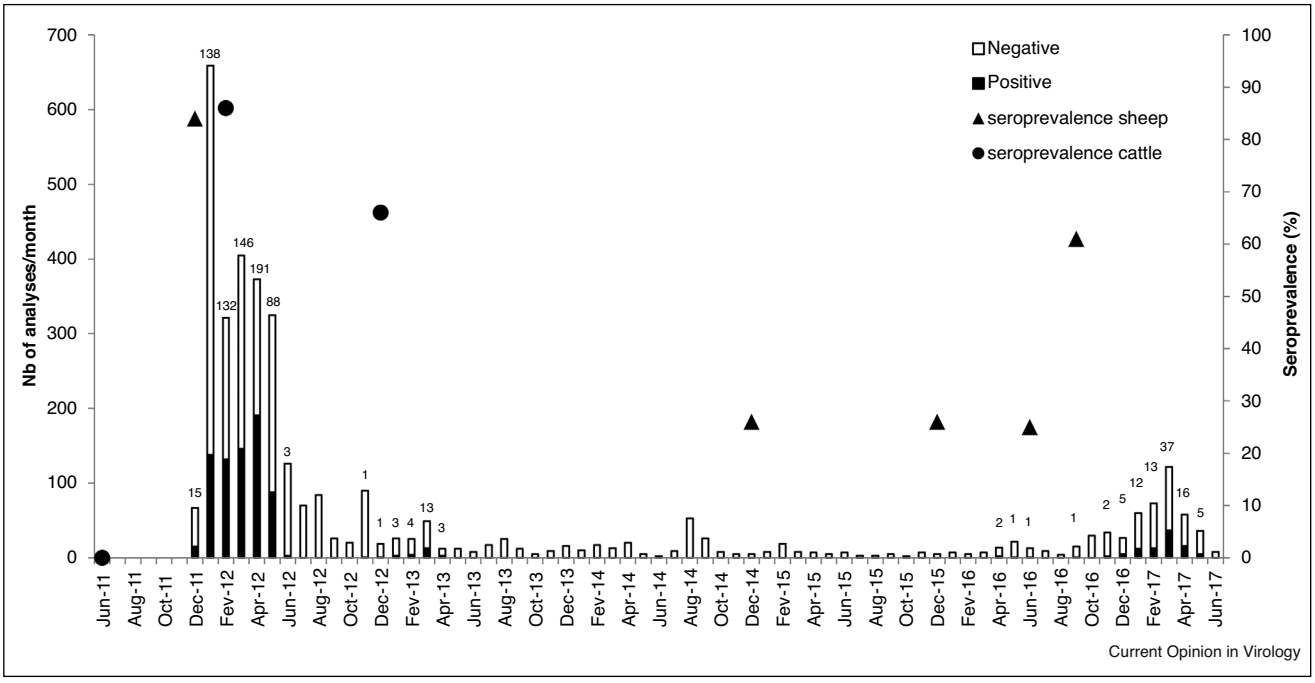
SBV was first identified using a metagenomics approach in plasma samples collected during the summer of 2011 from adult cattle with fever and reduced milk production originating from the city of Schmallenberg, Germany [25,26•]. The virus spread rapidly over Europe and its presence was reported by 29 European countries in a time span of less than 2 years, causing outbreaks of congenital malformation in winter and spring of 2011–2012 and 2012–2013 [27]. Since high levels of population immunity, reaching up to 90% and more, were reported after the initial spread of SBV through Europe [2], it was first unclear whether the virus would disappear due to a shortage of serologically naïve animals to sustain transmission, or would become endemic with potentially cyclic re-emergences associated with congenital malformations [28]. Data from several countries indicate that SBV has circulated endemically at a very low level since 2013 while the population immunity against SBV declined [12,29–33]. The intimate relationship between herd immunity and outbreaks of congenital malformations (Figure 1) is illustrated by epidemiological data from Belgium, showing that this decline in herd immunity was followed by a sudden increase in seroprevalence against SBV during the summer of 2016 (Figure 1). As could be expected, this resulted in a renewed outbreak of congenital malformations in the winter of 2017. The extent of the outbreak was however clearly lower than during the initial emergence in 2011 (Figure 1), probably because the remaining 25–30% of SBV-specific antibody positive animals regularly interrupted and prevented the exponential process of midge to host to midge cycling what normally causes the extensive spread of the infection. An increased SBV circulation in 2015 and 2016 was also reported in Germany, the Netherlands and the UK [34].

Vectors of AKAV, AINOV and SBV

Although AKAV and AINOV were initially isolated from mosquitoes, these seem not important for their transmission [35]. SBV was not detected in mosquitoes in different studies and *Culex pipiens* mosquitoes showed no vector competence for SBV under laboratory conditions [36–38].

Culicoides biting midges, small hematophagous flies belonging to the *Ceratopogonidae* family, have been implicated as the putative vectors for these three Simbu

Figure 1



The relationship between herd immunity against Schmallenberg virus and outbreaks of congenital malformations in Belgium. The figure plots the results of different nation-wide cross sectional seroprevalence studies performed in sheep and cattle in Belgium [29,114–118] in relation to the number of SBV suspected samples from ruminants monthly submitted to the Belgian reference laboratory CODA-CERVA for rRT-PCR diagnosis between November 2011 and June 2017. Although SBV has never been declared as a notifiable disease, samples were sent to us in the context of an existing mandatory notification of all aborted fetuses in cattle (abortion protocol) [119]. Numbers above the bars indicate the number of PCR positive animals detected.

viruses. Female *Culicoides* acquire the virus during a blood meal, necessary for the maturation of her eggs, on a viremic vertebrate host. The virus then replicates within the *Culicoides* and sometimes reaches the salivary glands. Replication in the salivary glands makes that new virus becomes available for transmission to a new host during a subsequent blood meal of the midge [39].

Depending on the geographical region, one or few species with high vector capacity seem to be responsible for most virus spread: *C. brevitarsis* in Australia, *C. oxystoma* in Japan, *C. imicola* in Israel and *C. obsoletus/scoticus* in Europe [40,41•,42,43]. The implication of *Culicoides* as vectors for AKAV, AINOV and SBV is mainly based on virus isolation from or detection in these species and on circumstantial epidemiological evidence that the distribution range and seasonality of these diseases match those of *Culicoides*. Compared to other *Culicoides*-borne viruses, e.g. bluetongue virus and African horse sickness virus, very limited thoroughly executed vector competence studies have been performed to irrefutably confirm the role of different *Culicoides* species as competent field vectors for the Simbu viruses. Only the oral susceptibility of *C. brevitarsis* for AKAV and *C. sonorensis* for SBV was reported [39,44] and AKAV was shown to replicate in *C. variipennis* after oral infection and transmission could occur after 7 to 10 days of incubation [45].

The seasonality of *Culicoides* distribution strongly depends on climatic conditions, and temperature and rainfall are the most determining factors [39]. In some tropical regions vectors are known to be present year round, like *C. brevitarsis* in northern Australia [46•], while in temperate climate regions, the number of adult *Culicoides* strongly decrease or even virtually disappear during the cold winter months [39]. These differences in *Culicoides* seasonality strongly influence the epidemiology of the disease caused by the Simbu viruses in different regions.

Adult clinical disease

Infection of adult cattle, sheep or goats with AKAV is usually not associated with clinical signs [35]. Some strains (e.g. Iriki strain) have however been reported to occasionally induce encephalomyelitis associated with nervous signs upon infection of postnatal cattle [47]. Infection of adult ruminants with SBV can be asymptomatic, but is regularly reported to be associated with fever, diarrhea and reduced milk production. These latter symptoms observed in German cattle led to the discovery

of SBV, even before the first congenital malformations were observed in the field [25]. Little data on this topic are available for AINOV. One report mentions the isolation of AINOV from a dairy cow with astasia [48].

Infection of adult ruminants with AKAV and SBV induces a short viremia (mostly between 2 and 5 days) within the first week after infection. Within 2–3 weeks after infection, neutralizing antibodies can be detected [2,35]. These protect the host against reinfection [34,49,50] and AKAV-specific and SBV-specific antibodies have been shown to persist in cattle for at least two and three years, respectively [30]. The protective immunity against SBV in sheep lasts at least for 16 months [50]. Neutralizing antibodies are passed to the progeny via colostrum and a limited number of reports indicate that they remain present for 4 and 5–6 months in calves for AKAV and SBV, respectively, and for up to 7 months in lambs infected with AKAV [51–53].

SBV has been detected in the lymphoreticular system of cattle, sheep and goats upon subcutaneous inoculation during experimental infections and was reported to be sporadically present in ovaries of infected sheep and goats [2,54,55•]. The relevance of these findings is currently unclear. Infectious SBV has also been detected in bovine semen from naturally infected bulls, but the actual risk of SBV transmission by insemination of dams with SBV-containing sperm however remains to be elucidated [56]. Whether infectious AKAV is present in sperm remains inconclusive [57–59].

Consequences of infection during pregnancy

The most eye-catching symptoms induced by AKAV, AINOV and SBV are the congenital malformations observed in newborns after infection of pregnant cattle, sheep and goats. After infection of the adult, the virus reaches the placenta via the blood, where it locally replicates, and subsequently can infect the developing fetus, sometimes leading to induction of abortion, stillbirth, birth at term of progeny with neurological signs and/or head, spine or limb malformations [28,35,60]. The different degrees of malformations in the brain, spinal cord, and skeletal muscles are often referred to as the 'arthrogryposis-hydranencephaly syndrome'. Macroscopic findings include varying degrees of arthrogryposis and different vertebral malformations like torticollis, kyphosis, lordosis and scoliosis. Pathological findings in the central nervous system vary from encephalomyelitis, porencephaly, hydranencephaly to microcephaly. Detailed descriptions of the pathological lesions are available [35,61,62] and were recently reviewed for AKAV, AINOV and SBV by Agerholm *et al.* [63].

Although only part of the progeny of infected dams is born with gross malformations, many studies tried to elucidate the conditions leading to their induction. These will be summarized in the paragraphs below. In general, the most susceptible period for induction of congenital malformations lies between the moment of establishment of the contact between the mother and the fetus (formation placenta) at around 30 or 31 days of gestation in cattle and sheep and the moment the fetus becomes immunocompetent *in utero* (65–70 days of gestation in lambs; 150 days of gestation in calves) [35,64].

Akabane virus

Experimental infections of pregnant cattle with AKAV and close follow-up of infected sentinel herds during outbreak periods (Table 1) showed that the type of abnormality induced by transplacental virus transfer is strongly influenced by the stage of gestation at which the dam is infected. AKAV infection during the first two months of pregnancy in cattle does not seem to have an impact on the fetus [18]. Infection between 76 and 104 days of gestation induces severe brain malformations like hydranencephaly and porencephaly. Calves with these lesions are often born alive but are considered as 'dummy' calves. They are usually capable of standing, but are often blind, depressed, poor responsive to external stimuli and without a strong sucking reflex. Infection between approximately 105–170 days of gestation leads to calves with arthrogryposis, combined or not with vertebral deformities like torticollis, scoliosis or kyphosis. Malformations resulting from infection during the last trimester of gestation, when immunocompetence is established, tend to be less severe and less common. Such calves often have difficulties standing and are uncoordinated and ataxic, but are usually bright and alert and have a good sucking reflex. These symptoms can mostly be related to a non-suppurative encephalomyelitis. Infection late in gestation can also induce abortions and stillbirths, which sometimes appear grossly normal [18,35]. It is important to emphasize that this division of pregnancy in periods with different sensitivities for the induction of malformations is indicative but not absolute, making that it is not always possible to reliably estimate the stage of gestation at which the fetus was infected based on the presentation at birth [35]. Furthermore, not each infection during the sensitive period of gestation leads to the birth of malformed progeny [35].

All different malformations found in cattle have also been observed in progeny of AKAV infected pregnant sheep and goats (Table 1). The chronological progression of defects as observed in cattle is however not usually apparent, probably due to the shorter gestation period in sheep (±150 days) compared to cattle (±280 days) [35]. Inoculation with AKAV between day 29 and 50 of gestation seems most effective in reproducing congenital malformations, with most severe lesions observed after infection between day 30 and 36 of gestation [60].

Table 1

Overview of experimental and field studies with Akabane virus, Aino virus and Schmallenberg virus in domestic ruminants contributing to our understanding of the efficacy of transplacental crossing, the capacity to induce congenital malformations, and the relationship between clinical and pathological malformations observed at birth in offspring and the moment of gestation at which the dam was infected

Reference	Study type		Virus			Strain	Species[a]			Number studied in detail	Inoculation route	Moment infection (day gestation)	Moment progeny was studied	Major outcome
	XP	field	AKAV	AINOV	SBV		C	S	G					
Kurogi *et al.*, 1976 [109]		x	x			Field strain	x			2	Vector-mediated	?	92 and 134 dg	Isolation of OBE-1 strain from fetus of naturally infected dam
Kurogi *et al.*, 1977 [120]	x		x			OBE-1	x			11	Intravenous	62–96	18 dpi or at birth	Evidence AKAV is etiological agent for abortions and malformations
Hartley *et al.*, [98]		x	x			Field strain	x			130	Vector-mediated	?	Birth	Classification of lesions in five groups corresponding to infection at different stages of gestation
Coverdale *et al.*, 1978 [68]		x	x			Field virus	x			25	Vector-mediated	?	Birth	Hydranencephaly in dummy calves
Konno *et al.*, 1982 [99]		x	x				x			177	Vector-mediated	?	Birth	Classification of lesions in three groups probably corresponding to infection at different stages of gestation
Kirkland *et al.*, 1988 [22]		x	x			Field strain	x			174	Vector-mediated	0–80	Birth	Observed lesions are related to stage of gestation at moment of infection
Kitano *et al.*, 1994 [121]		x	x			Field strain	x			17	Vector-mediated	?	0–155 days after birth	17 dummy calves with hydranencephaly without precolostral AKAV-specific antibodies
Uchida *et al.*, 2000 [122]		x	x			Field strain	x			27	Vector-mediated	?	1–27 days after birth	Calves at start from outbreak without antibodies but with encephalomyelitis
Lee *et al.*, 2007 [123]		x	x			Field strains	x			7	Vector-mediated	?	3–17 days after birth	Calves with encephalomyelitis without neutralizing antibodies
Noda *et al.*, 2001 [124]		x	x			Field strain	x			8	Vector-mediated	?	2–20 days after birth	Ataxic calves with encephalomyelitis were IHC positive in brain
Brenner *et al.*, 2004 [125]		x	x			Field strain	x			n.r.	Vector-mediated	Unknown	After birth	Blind calves with hydranencephaly in 2002 in Israel
Brenner *et al.*, 2016 [20•]		x	x			Field strain	x			20	Vector-mediated	?	Shortly after birth	Apparently healthy neonates PCR positive in blood

Table 1 (*Continued*)

Reference	Study type		Virus			Strain	Species[a]			Number studied in detail	Inoculation route	Moment infection (day gestation)	Moment progeny was studied	Major outcome
	XP	field	AKAV	AINOV	SBV		C	S	G					
Parsonson *et al.*, 1975 [126] Parsonson *et al.*, 1977 [127•]	x		x			B8935		x		25	Intravenous	28–82	At birth	Only congenital malformations induced in ewes inoculated between 30 and 36 days of gestation
Della-Porta *et al.*, 1977 [128]		x	x			Field strain		x		9	Vector-mediated	Not known	72–89 dg	AKAV isolation from malformed fetuses from sacrificed ewes identifies the virus as etiological agent
Hashiguchi *et al.*, 1979 [129•] Narita *et al.*, 1979 [130]	x		x			OBE-1		x		35	Intravenous	29–101	Partly between 9 and 30 dpi and partly at birth	AKAV crossed placenta efficiently when inoculated between 29 and 45 dg and induced malformations at birth; little evidence of placental crossing and induction of malformations is found when ewes are infected later that 81 days of gestation
Parsonson *et al.*, 1981 [100,131]	x		x			CSIRO16		x		39	Intravenous	32-36	69–105 dg	AKAV isolated from blood of fetuses sacrificed at 95–106 dg and frequent isolation of AKAV from placentomes
Parsonson *et al.*, 1988 [57]	x		x			CSIRO16		x		36	Intravenous	32–33	46–53 dg	AKAV replicates in placenta 1dpi and in fetus at 5 dpi, mostly in brain and to lesser extent in skeletal muscles; placenta remains physiologically intact despite high titers of AKAV
McClure *et al.*, 1988 [106]	x		x			CSIRO16		x		42	Partly intravenous; partly intra-uterine	31–126	40–135 dg	Absence of cell mediated immune response in fetuses of ewes inoculated between 31 and 44 dg
Kurogi *et al.*, 1977 [132]	x		x			OBE-1 and JaGAr39			x	10	Intravenous	30–115	40 dg and birth	Transplacental passage occured based on virus isolation from fetuses at 10 dpi, but only one fetus with gross malformations
Parsonson *et al.*, 1982 [60][b]	x			x				x				90		No congenital malformations induced
Tsuda *et al.*, 2004 [19•]	x			x		KSB-3/P/95	x			5	Intravenous	122–162	At birth	No proof of transplacental infection

Table 1 (*Continued*)

Reference	Study type		Virus			Strain	Species[a]			Number studied in detail	Inoculation route	Moment infection (day gestation)	Moment progeny was studied	Major outcome
	XP	field	AKAV	AINOV	SBV		C	S	G					
										5	*In utero* — intraperitoneal	132–156	At birth	When placental crossing is bypassed, efficient induction of malformations
Schmallenberg virus: final report EU, 2014[d]	x				x	FLI inoculum[c]	x			24	Subcutaneous	60–150	6 weeks pi	Preliminary data; indications for efficient placental crossing but limited capacity to induce malformations
Schmallenberg virus: final report EU, 2014[d]	x				x	FLI inoculum[c]	x			11	Subcutaneous	105–120	10–28 dpi	Preliminary data; indications for efficient placental crossing but limited capacity to induce malformations
Wernike *et al.*, 2014 [77•]		x			x	Field strain	x			71	Vector-mediated	13–162	At birth	Evidence of transplacental SBV infection only found in 13% of calves at birth and only 1 calve with malformations
Schmallenberg virus: final report EU, 2014[d]	x				x	FLI inoculum[c]		x		21	Subcutaneous	38 and 45	7 dpi	Preliminary data; indications of transplacental crossing in 64% of fetuses at 7 dpi; no malformations observed
Martinelle *et al.*, 2015 [55•] Poskin *et al.*, 2017 [107•]	x				x	FLI inoculum[c]		x		17	Subcutaneous	45 and 60	At birth	Evidence of transplacental SBV infection only found in 14% of lambs at birth; no congenital malformations observed; placenta of 5 ewes contained infectious SBV at birth
Schmallenberg virus: final report EU, 2014[d]	x				x	FLI inoculum[c]			x	10	Subcutaneous	28 and 42	14–25 dpi	Preliminary data; several hemorhagic and small fetuses observed after SBV infection
Steinrigl *et al.*, 2014 [75]		x			x	Field strain	x	x	x	13	Vector-mediated	32–81		13 cows with early fetal death after SBV infection

Manuscripts found via the use of search terms 'Akabane virus', 'Aino virus', 'Schmallenberg virus' in databases 'Web of Science' and 'Pubmed' and available to the author via libraries of VDIC and Ghent University are mentioned.

[a] C = cattle; S = sheep; G = goat.

[b] Original manuscript not available to the author, results mentioned in [60].

[c] Inoculum consisting of bovine serum collected at 3d post SBV infection, prepared and distributed by the Friedrich Loeffler Institute.

[d] Schmallenberg virus, March 2014, Technical and scientific studies, Final report for European Union Commission Implementing Decision of 27 June 2012: accesible online at library.wur.nl/WebQuery/wurpubs/fulltext/310772.

dg: days of gestation; dpi: days post infection.

Only limited information is available on the impact of AKAV infection on herd or population level. In a field study with 174 cows in a year-round calving herd, Kirkland *et al.* [65•] reported that malformed calves were found in 18% of pregnant cows. Inaba *et al.* [66] mentioned that almost 42 000 cases of abortion, stillbirth and congenital arthrogryposis-hydranencephaly were reported in cattle during the outbreak of 1972–1975 in Japan. Haughey *et al.* [53] reported that lamb mortality due to congenital AKAV infection varied between 5%and 92% on four sheep farms during an outbreak in 1976 in Australia.

Aino virus

It was only after the outbreak of 1995–1996 in Japan that AINOV was detected via immunohistochemistry and virus isolation in a fetus aborted from a naturally infected cow [24,67]. Before, Aino was considered as an etiological agent of congenital malformations based on the detection of Aino-specific antibodies in precolostral sera of malformed calves [68]. Congenital malformations could not be reproduced in an experimental infection study in sheep [60] but this might be related to the fact that sheep were only inoculated at day 90 of gestation. Also no proof of transplacental transmission was found after experimental intravenous AINOV infection of pregnant cows between 4 and 5 months of gestation [19•]. Only when the placental barrier was bypassed via an intra-uterine intraperitoneal inoculation in the fetus, severe malformations in the fetuses were induced [19•]. It can therefore be concluded that AINOV has a more limited capacity to cross the placenta than AKAV.

Schmallenberg virus

Starting from the end of November 2011 onwards, an epizootic outbreak of abortions, stillbirths and congenital malformed lambs, calves and goat kids was reported by several European countries [2,61,62,69] and SBV could be incriminated as the etiological agent in part of the suspected cases by real time PCR detection of SBV RNA in different fetal or neonate tissues and/or by the presence of SBV-specific antibodies in precolostral serum or body fluids [70–72]. Most research focused on the offspring with the typical symptoms of the arthrogryposis-hydranencephaly syndrome and similar cerebrospinal and musculoskeletal malformations as described above for AKAV infection were rapidly reported. To a lesser extent, also live born, blind and depressed 'dummy' lambs and calves and calves only showing encephalomyelitis with associated neurological symptoms but without gross lesions were reported [61,73–75]. Altogether, this provides strong indications that a similar correlation between the induced abnormality and the stage of gestation at which dams are infected exists for SBV as for AKAV, but data are currently not conclusive since information on the exact moment of SBV infection is missing in most studies. Interestingly, in contrast to AKAV, data in cattle is available suggesting that SBV infection during early gestation can lead to early embryonic death and return of the dams to oestrus [75,76].

So far only a limited number of experimental infection studies in pregnant cattle, sheep and goats have been reported and some data are only preliminary (Table 1). The available data suggest that SBV is relatively efficient in crossing the ruminant placenta and reaching the fetus since SBV RNA was often (mostly in more than 50% of the fetuses) detected by real time PCR in fetuses of calves, lambs and kids shortly after SBV infection of the dams. The virus however seems to be rapidly eliminated from the fetus in most cases, without the induction of antibodies or malformations. In the only experimental study wherein ewes were allowed to give birth at term after infection at 45 and 60 days of gestation, no malformations were found and proof of SBV transplacental infection was only found in 3 out of 21 lambs, while embryonic structures of 13 of the 21 lambs were still SBV RNA positive in PCR [55•]. The only field study in which all pregnant cows and their offspring of a well-defined herd were followed also indicates a limited capacity of SBV to induce malformations [77•]. Only one malformed calf was born from 71 cows infected between 0 and 162 days of gestation but samples were unavailable to confirm the involvement of SBV. Only in 9 of the 70 other 'normal' calves, evidence of transplacental virus infection could be found by the presence of SBV-specific antibodies in precolostral serum or SBV RNA in meconium. This limited efficiency of SBV to induce congenital malformation seems in line with data indicating that the impact of the emergence of SBV in 2011–2012 was in fact limited at population level [27,76,78,79]. In some sheep herds, however, the impact of SBV was extensive with more than 50% abnormal births [78].

Role of domestic ruminant hosts and *Culicoides* vectors in virus transmission and overwintering

Common transmission of AKAV, AINOV and SBV between infected and susceptible ruminant hosts occurs by hematophagous female *Culicoides* that acquire the virus during a blood meal on the infected, viremic host. After an extrinsic incubation period associated with virus replication in the vector, the virus is passed to the susceptible host during a next blood meal. Persistence of these Simbu viruses in a certain region thus depends on the presence of vectors and susceptible hosts in close proximity [60].

In tropical endemic regions with year-round vector activity, for example, northern Australia [46•], it is albeit possible that the virus cycling between competent vectors — susceptible hosts — competent vectors sustains

continuous virus circulation. Nevertheless, also in such tropical and subtropical regions, a tendency to seasonal virus transmission is noticed with highest infection rates found in moist summer months and lower transmission rates during dry winter periods [18,39].

Regular presence of AKAV, AINOV and SBV and associated cyclic outbreaks of congenital malformations have however also been reported from temperate climate regions (see above), where virus circulation is interrupted during cold winter periods due to the absence or only very limited presence of vectors. This timely absence of vectors leads to a seasonal pattern of virus transmission, with highest infection rates found in warm and moist summer and autumn months, coinciding with highest vector abundance [39,80,81•,82].

Interestingly, very little information is currently available to explain how AKAV, AINOV and SBV cross-pass the yearly periods of low vector abundance in temperate climate zones. The following paragraphs will summarize hypotheses that have been put forward to explain the overwintering of these viruses and introduce some alternative ones based on indications mostly found in recent literature.

Yearly reintroduction from endemic regions

A first hypothesis to explain the virus overwintering is the occurrence of yearly virus reintroductions via wind-borne spread of infected midges or via movement of infected hosts from tropical endemic zones into the temperate climate regions [39]. Kato *et al.* [41•] indicate that such yearly reintroductions via seasonal winds are the most likely explanation for the presence of AKAV in southern Japan, rather than a local persistence, since viruses could only be isolated from *Culicoides* collected after July while indigenous *Culicoides* are already present from May onwards and since they never isolated the same viruses in consecutive years. A similar mode of action could also occur in Australia via wind-borne introductions from endemic northern regions to more temperate southern regions [46•].

Although plausible for temperate climate regions located relatively close to known tropical regions with endemic virus circulation, this hypothesis seems less applicable to explain the observed presence of SBV in western European countries which lie in the temperate climate zone. No SBV endemic (sub)tropical regions are currently known that could accommodate yearly reintroductions and several reports indicate that a constant low endemic SBV circulation occurred since the initial emergence in several countries [12,29–33], even during the winter months of 2011–2012 [62,83], being rather suggestive for a local persistence. Other ways of virus overwintering thus probably apply to SBV in Europe.

Overwintering in the vector

Adult Culicoides

The most supported hypothesis to explain SBV overwintering in Europe is the persistence of the virus in adult *Culicoides* that survive winter [84]. This is based on studies showing that low numbers of adult midges can be found during winter in temperate regions [85–88] and seems in line with the detection of SBV circulation in cattle during winter 2011 in Germany at intermittent moments of increasing temperatures [83]. A similar mechanism of overwintering in surviving adult *Culicoides* has been postulated for bluetongue virus in California [89] and African horse sickness virus in South-Africa [90].

In opposition hereto, many European countries report long vector-free periods (often from December to March/April; <5 parous female *Culicoides*/collection) in the context of the EU regulation 1266/2007 to loosen trade and movement restrictions imposed to countries with bluetongue virus circulation [91], indicating that the active *Culicoides* population during winter in Europe is very low. Recent reports even show complete vector-free periods lasting for several winter months inside and outside horse, cattle and sheep stables in Germany and Austria [84,91]. Some estimate that the limited number of adult midges surviving winter cannot be sufficient to keep virus circulation ongoing to the next vector season and advocate that alternative pathways must occur [41•,92]. One of those could be transmission via alternative vectors such as ticks or biting flies but no evidence therefore is available [92].

Transovarial transmission

Another potential overwintering strategy that received some attention is the vertical passage of viruses in *Culicoides* via transovarial transmission (TOT), in analogy with observations made for some viruses transmitted by mosquitoes [93]. One study was not capable to confirm the occurrence of transovarial transmission of AKAV under field conditions in Australia [94]. Larska *et al.* [95] suggested that TOT might occur for SBV based on SBV RNA detection via PCR in midges considered to be nulliparous based on visual examination but no reports followed to confirm this hypothesis. In Belgium, no SBV RNA was detected in 1359 nulliparous female midges collected in May 2012 at places where SBV had circulated in 2011 [82]. Transovarial transmission has neither been reported so far for the more intensely studied bluetongue virus [96].

Overwintering in infected hosts, progeny, or placental tissues

Postnatal ruminant hosts

As described above, the viremic period in AKAV, AINOV or SBV infected postnatal ruminants only lasts for a few days. Therefore, it seems highly unlikely that these infected hosts can serve as virus reservoirs that transfer these viruses toward the next vector season.

Since this review focuses on AKAV, AINOV and SBV infections in cattle, sheep and goats, the role of wildlife and other host species was not assessed. The frequent detection of antibodies against AKAV and SBV in multiple wildlife species suggests however that wildlife can currently not be excluded a potential reservoirs of these viruses [97].

Transplacentally infected neonatal progeny

Until now, a potential role for vertical transplacental virus transmission in the ruminant host has never been seriously considered as a potential mechanism whereby AKAV, AINOV or SBV could overwinter the cold season.

A first group to consider is the offspring with congenital malformations. These were studied most intensively and it is reported that detection of AKAV and SBV by virus isolation is very difficult in this group [10,34,98–101]. Together with the finding that also SBV RNA was often no longer detectable by real time PCR in malformed progeny at birth [61,71,72], this largely explains why this group is not considered as a potential source of virus for newly emerging adult *Culicoides* during next spring. The cumbersome virus detection at birth is probably the result of the long gestational period during which virus can be gradually eliminated from the fetus and the presence of neutralizing antibodies that mask infectious virus or contribute to virus elimination during gestation [2,71,98–100].

A second group are the neonatal lambs and calves born without severe gross malformations that are mostly observed during the beginning and end of the outbreak seasons of AKAV and SBV. These show different degrees of neurological symptoms, and involvement of AKAV or SBV infection can often be found during autopsy or using diagnostic techniques. These have been studied in far lower numbers than malformed newborns. Several reports are however available mentioning that some of these newborns lack the presence of virus-specific neutralizing antibodies while hints for potential virus presence were found in some of them (Table 2). Although none of the observations is currently conclusive in showing that these neonates contain sufficient levels of infectious virus in the absence of neutralizing antibodies that would allow *Culicoides* to become infected during a blood meal, they clearly support manuscripts stating that this group of progeny needs to be studied more intensively to properly evaluate their potential role in virus persistence [75,102].

A third group of progeny born from AKAV or SBV infected dams that theoretically could occur and play a role in bridging virus into the next vector season would be apparently healthy but immunotolerant persistently infected (PI) newborns. This phenomenon has been described for pestiviruses as bovine viral diarrhea virus and border disease virus [103] and the midge-borne bluetongue virus serotype 8 [104] and seems to occur in 1–2% of newborns. It can occur when a fetus is *in utero* exposed to these viruses before the maturation of the immune system, making that the virus is recognized as self and resulting in an immune-tolerant state and persistent viremia without seroconversion in the newborn [105]. Such PI animals are not believed to occur upon AKAV and SBV infection of pregnant dams [35,77•] but no studies are present in literature that have addressed this in sufficient detail to formally exclude this option. Only some indirect indications are found in immunological studies that support a potential occurrence of persistently infected animals upon transplacental passage of AKAV and SBV [69,106].

Placenta harboring infectious virus

Another potential hypothesis to explain SBV overwintering has recently been raised after infectious SBV was found in fetal envelopes collected at birth of 1/8 and 4/9 ewes (mistakenly reported as 1/5 and 4/6 in the original manuscript [107•]) that were experimentally infected with SBV at 45 and 60 days of gestation, respectively [55•,107•]. This shows that placenta represents a source of infectious virus that becomes available at more than 100 days after the initial infection of the ewe. It will be most interesting to verify whether similar SBV, and potentially AKAV, persistence also occurs in fetal envelopes of pregnant cattle, since this would allow the virus to bridge even longer time periods and become available in spring when new adult *Culicoides* are already emerging [80,108]. The latter seems plausible based on observations that placenta is considered as one of the best diagnostic samples for SBV RNA detection by real time PCR at birth of malformed offspring [70]. Also for AKAV the placenta is considered as an immunologically privileged site where virus persistence occurs [35] and AKAV was detected by virus isolation in placenta of ewes at 100 days of gestation which were infected 65 days earlier [100]. Reports in cattle are limited to virus isolation from placenta at around 20 days post infection [109] but no evaluations later in gestation or at birth have been described.

The most challenging question related to these results is how the persisting virus present in placentomes could restart virus transmission at the beginning of a new vector season. One hypothesis could be that unprotected seronegative dams could become infected after eating infected placenta. This seems opposed by studies showing that oral inoculation of two cows and three sheep with SBV containing fluids did not result in a productive infection [49,110] and that dams eating their own infected placenta are supposed to be protected by neutralizing antibodies resulting from the primary infection. It should however be considered that much higher amounts of virus could be present in a placenta compared to what was orally administered before ($10^{5.5}$ $TCID_{50}$/g placenta was

Table 2

Reports of virus-specific antibody negative offspring without gross malformations at birth observed during experimental and field studies with Akabane virus and Schmallenberg virus in domestic ruminants

Reference	Virus	Species	Study type		Case presentation		Estimated moment infection (gestation)			Antibody status	Virus status	Missing information
			XP	Field	Clinical	Autopsy	Early	Mid	Late			
Parsonson *et al.*, 1975 [126]	AKAV	Sheep	x		Apparently healthy	Porencephaly	x			Negative	n.t.	Virus isolation from blood
Parsonson *et al.*, 1977 [127•]	AKAV	Sheep	x		Apparently healthy	Porencephaly	x			Negative	Virus isolation negative	
Hartley *et al.*, 1977 [98]	AKAV	Cattle		x	Incoordination; some blind	Porencephaly hydranencephaly micrencephaly	x			Negative	Virus isolation negative	
Kurogi *et al.*, 1977 [132]	AKAV	Goat	x		Normal but weak	n.r.		x		Negative	n.r.	Virus isolation from blood
Hashiguchi *et al.*, 1979 [129•] Narita *et al.*, 1979 [130]	AKAV	Sheep	x		Apparently healthy	Cyst formation	x			Negative	n.t.	Virus isolation from blood
Kitano *et al.*, 1994 [121]	AKAV	Cattle		x	Dummy calves	Multiple brain defects	x			Negative	Virus isolation negative	Virus isolation from blood
Uchida *et al.*, 2000 [122]	AKAV	Cattle		x	Ataxia	Encephalomyelitis			x	Negative	IHC positive in brain	Virus isolation from blood
Noda *et al.*, 2001 [124]	AKAV	Cattle		x	Ataxia	Encephalomyelitis			x	Negative	IHC positive in brain; virus isolation negative	Virus isolation from blood
Lee *et al.*, 2007 [123]	AKAV	Cattle		x	Ataxia	Encephalomyelitis			x	Negative	n.t.	Virus isolation from blood
Wernike *et al.*, 2014 [77•]	SBV	Cattle		x	Apparently healthy	n.r.		x		Positive	PCR positive in serum	Virus isolation from blood
Peperkamp *et al.*, 2015 [62]	SBV	Sheep		x	n.r.	Encephalomyelitis			x	Negative	PCR and IHC positive in brain	Virus isolation from blood
Brenner *et al.*, 2016 [20•]	AKAV	Cattle		x	Apparently healthy	n.r.	?	?	?	n.t.	PCR positive in blood	Virus isolation from blood. Presence of antibodies

n.r. = not reported; n.t. = not tested.

found in placenta of AKAV infected sheep at 100 days of gestation [100]), and that under field conditions, also naïve dams can eat the placenta of another, potentially infected dam. The latter occurred in a study on bluetongue virus transmission and provoked a viremia in the heifer eating the placenta [111]. If something similar would occur for SBV or AKAV, this could give rise to a new viremic host that then serves as a starting point for further virus transmission.

Another hypothesis could be that placentas containing infectious virus could serve directly or indirectly as a food source for overwintering *Culicoides* larvae after being discarded on dung heaps, a preferred *Culicoides* larval habitat [112]. These could then potentially pass the virus through further developmental stages, leading to infected adults. Finally, the incomplete knowledge of *Culicoides* biology also does not allow to completely exclude that such placenta could directly be used by adult midges as a food source to accommodate their need for carbohydrates [113], resulting in simultaneously ingestion of virus that could potentially give rise to infected adult vectors.

Remarks

Some hypotheses raised above are rather unconventional and arguments can be raised making some of them very unlikely, but the point remains that the current knowledge on *Culicoides* biology and Simbu virus pathogenesis in some groups of neonatal progeny is too limited to formerly exclude them. More research on *Culicoides* biology and studies focusing on healthy newborns without gross malformations born from AKAV, AINO and SBV infected dams will have to shed light on this matter.

It is important to consider that also rare or inefficacious occurrences or mechanisms could be of importance given the high abundance of *Culicoides* midges and the high number of pregnant dams that become infected at various stages of gestation when AKAV, AINOV or SBV is introduced in a naïve or only limited protected host population. Sporadic occurrences might be sufficient to keep virus circulation going at a low level and assure the presence of virus when appropriate climate conditions, susceptible hosts and competent vectors become available.

Conclusions

AKAV, AINOV and SBV are three viruses belonging to the Simbu serogroup of Orthobunyaviruses capable of inducing congenital malformations upon infection of cattle, sheep and goats. Available experimental and field data show that they have many resemblances, like dependence on *Culicoides* vectors for their transmission and the necessity to get infected during specific stages of gestation to induce malformations. Differences seem however present in their potential to induce clinical disease in adults and early embryonal death and in their efficacy to cross the placenta and to induce malformations.

Since AKAV and AINOV are present in different geographical regions than SBV, they seem to use different mechanisms to cross-pass periods with low vector activity. This aspect has however only been studied to a limited extent so far and more work on *Culicoides* biology and specific groups of transplacentally infected progeny will be necessary to shed light on this matter. In addition to already postulated mechanisms to explain this phenomenon, some unconventional hypotheses were added along this review.

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Conflict of interest

None declared.

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- • of special interest
- •• of outstanding interest

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