Schmallenberg virus (SBV)

The use of semen from SBV-seropositive donor bulls

Contents:

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1. <u>Epidemiology:</u>

The virus

Schmallenberg virus (SBV) belongs to the Simbu serogroup viruses within the Bunyaviridae family and the Orthobunyavirus genus, as do Akabane virus (present a. o. in Japan, Australia) and Cache Valley Disease (USA). Members of this family of viruses are found worldwide and affect both humans and animals. SBV, however, is not zoonotic (it cannot spread from animals to humans).

Schmallenberg virus is arboviral, i.e. it is transmitted by insects (Culicoides), and it must replicate itself in the salivary glands of its intermediate host to become virulent.

The main hosts are domestic or wild ruminants (bovine, ovine, caprine and bison). Infected adult animals usually show only mild, general symptoms, but the virus poses a risk for offspring when infected during a certain period of time during pregnancy.

The Bunyaviridae family currently accounts for more than 350 viruses capable of infecting humans, animals and plants (Guu et al., 2012). Several viruses, including Cache Valley, Lokern, Main Drain, Northway, Potosi and Tensawm are present on the American continent, from Alaska to Argentina (Calisher, 1994; Sahu et al., 2002). Antibodies have been detected in sick or asymptomatic domestic mammals (pigs, bovine animals, sheep and goats) and wild mammals (caribou, deer, White-tailed deer) (Campbell et al., 1989; Neitzel and Grimstad, 1991; Blackmore and Grimstad, 1998; Nagayama et al., 2001). For other Bunyaviridae, but not for SBV, also in humans, with a few rare cases of encephalitis or meningitis (Sexton et al., 1997; Sahu et al., 2002; Campbell et al., 2006). Cases of congenital malformations (Arthrogryposis – Hydranencephaly) linked to Cache Valley virus (CVV) were reported in sheep in Texas, USA, in late 1986, then in other States, including North Dakota, Pennsylvania, Michigan and Nebraska (Crandell et al., 1989; Edwards et al., 1989; Edwards, 1994). The virus has also been detected in young calves suffering from the same malformations, in Canada in 1975 (Calisher and Sever, 1995). Main Drain virus (MDV) led to the appearance of congenital malformations in experimentally-infected sheep (Edwards et al., 1997).

Geographical spread

Some cases of Orthobunyavirus infection have been previously reported in Europe, but no virus from the Simbu serogroup was isolated in Europe prior to 2011.

Schmallenberg virus was first detected in Germany in November 2011, in samples from sick dairy cows (fever and reduced milk yields) collected in summer and autumn 2011. Traditional clinical signs (especially diarrhoea) have been observed in dairy cows in the Netherlands, where the presence of Schmallenberg virus was also detected in December 2011.

Since early December 2011, congenital malformations have been reported in new-born lambs in the Netherlands. To date, cases of PCR-positive stillbirths and congenital malformations have been reported in the Netherlands, Belgium, Germany, UK, France, Luxembourg, Spain, Italy, Switzerland, Austria and Ireland. The spread of Schmallenberg virus has also been reported in numerous other countries.

Transmission

The virus is transmitted by biting insects taking blood from a carrier animal. To become virulent, the virus reproduces in the salivary glands of the intermediate host.

In-utero transmission by the mother to the foetus is also commonplace. Direct transmission by animal-to-animal contact has not been proven and remains unlikely.

Transmission via insemination with semen from seropositive animals has not been demonstrated.

2. The OIE and European Commission positions:

In 2013, the OIE published a technical factsheet on SBV (updated in June 2017, see http://www.oie.int/fileadmin/Home/eng/Our_scientific_expertise/docs/pdf/A_Schmallenberg_virus.pdf). Schmallenberg virus is not considered to be an OIE listed disease or a zoonosis.

The European Commission considers that it should not impose any trading restrictions and has repeatedly called upon Third countries to follow the same approach.

3. Screening tests:

The virus can be detected by RT-PCR testing in various biological samples, including blood and semen. The test was initially perfected by the German reference laboratory FLI: (Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health, Greifswald, Insel Riems, Germany). It was replicated and validated by different laboratories in EU Member States in a European inter-laboratory trial (ring test) with 44 analytical laboratories or research institutes, and demonstrated its resilience in managing viral RNA extraction methods. The technique has been described and documented and its protocol published in an international journal (Schulz et al, 2015).

Serological tests identifying antibodies that demonstrate a previous contact between the animal and the virus are:

- serum neutralisation test (SNT)
- enzyme-linked immunosorbent assay (ELISA)

a) Strategy employed by semen collection centres:

Animals being processed for semen: systematic serological screening of serologically negative animals every 28 days. When a previously seronegative donor is found to have seroconverted, all ejaculates from that bull produced since the previous negative serological test are PCR-tested to detect any containing viral RNA and any ejaculates giving positive results are destroyed.

Bulls resuming production after a lay-off period systematically undergo serological screening before semen collections in order to determine their status.

4. **Excretion of the virus in semen:**

Following infection, the viraemia lasts an estimated 5-7 days, sometimes less.

The virus can be excreted in the semen of a small percentage of infected bulls, not only during the viraemic period but also for a period of a few weeks after seroconversion. Excretion can be continuous or intermittent and its duration varies according to the individual animal.

Identifying the viral RNA in semen does not prove that the virus present is virulent and pathogenic and can subsequently transmit the disease via insemination and there is currently no evidence of females being infected by semen from seropositive bulls.

To prove definitively whether or not infected semen can infect inseminated females could require an experimental transmission trial to be carried out in controlled conditions. In fact, all the major semen collection companies consider it unnecessary to carry out such a trial. Even if it showed that infected semen could infect an inseminated cow, it would have no impact on trade because, by screening all ejaculates from seropositive bulls for the presence of virus, it can be ensured that infected semen is never used.

The principle of testing semen for diseases which are known to transmit in semen is well established, perhaps the best known example being Infectious Bovine Rhinotracheitis (IBR). It is known that IBR-seropositive bulls can excrete IBR virus in their semen which is capable of infecting an inseminated cow. However, many countries, including major importers such as USA, accept such semen provided it has tested negative for IBR virus. The same principle should apply to SBV.

In practice, all ejaculates from SBV seropositive bulls are RT-PCR tested before being distributed for export, even if the recipient countries have no SBV-related import requirements and only ejaculates displaying negative RT-PCR results are exported.

5. **Categories of semen available, based on donor health status** (as defined in supplementary SBV compliance documents like "additional declarations", AD)

- Semen produced before June 2011: the virus had not been detected in Europe at this time and use of such semen therefore presents no risk of infecting cows inseminated with it.

- Semen from seronegative bulls: donor status is determined by systematic serological analysis. While the animal continues to test seronegative, it is considered to be free of SBV infection and its semen is considered to present no risk of infecting cows inseminated with it. In practice, the period of semen production is overseen by serological tests, the latest of which is undertaken between 21 (28 days in certain cases) and 60 days after the last collection.

- Semen from seropositive bulls, or those of unknown serological status: all ejaculates destined for export are RT-PCR tested. Those displaying traces of viral RNA (positive) are destroyed.

6. **Usage of semen from SBV-seropositive bulls:**

At the time of writing, 13 countries to which bovine semen is exported from one or more of the EU member states (EU-28) will only accept semen from SBV seronegative bulls. These countries are listed in part a) of the table attached (pages 7 and 8), which shows the number of straws exported to those countries by the EU 28 during the six full calendar years since SBV was first identified, i.e. 2012 to 2017 inclusive.

Another 13 countries will accept semen from SBV-seropositive bulls, subject to it having tested negative by PCR. These are listed in part b) of the attached table together with the figures for semen exports in the same 6 years.

Part c) of the table lists countries, and the semen exports to them, which have no SBV-related import conditions. However, as noted above, all ejaculates exported to those countries from SBV-seropositive bulls will have been tested by PCR with negative results.

The table shows that a total of 60.3 mln doses of semen were exported by the EU 28 in the six years in question to other countries in the world. 17.3 mln doses (28.4%) were exported to the 13 countries in part a) and were therefore either from seronegative bulls or collected before 1 June 2011.

Of the remaining 43 mln doses (71.6% of the total), it is not possible to state how many came from SBV-seropositive bulls. Bearing in mind that all the major exporting countries, excluding Scandinavia, have experienced several waves of SBV infection during those six years and during which a big majority of bulls at the semen collection centres seroconverted, it is likely that a substantial proportion of those 43 million doses came from seropositive bulls. If it was only 25%, considered by the semen companies to be a very low estimate, that means that over 10 million doses of semen from seropositive bulls have been exported to countries that had not reported the presence of SBV in their countries and yet there has not been a single report of SBV infection occurring following the use of that semen!

The semen companies consider that this presents powerful evidence that, even if the presence of SBV virus in semen could cause infection in the inseminated cow, the use of the PCR test to screen the semen prior to use has proved to be highly effective. Therefore, Third countries should accept tested semen from seropositive bulls.

7. Summary

- SBV is a member of a family of viruses represented across the whole world (approximately 350 species).
- It is an "arboviral" vector-borne disease; the virus is transmitted by an intermediate host (Culicoides), in the salivary glands of which it reproduces to become virulent.
- The virus can be detected in semen in the days after infection and subsequent seroconversion, but the way it is excreted and persists varies according to individual animals, from continuous to intermittent and from a few days to a few weeks.
- Reliable tests, both serological (serum neutralisation, ELISA) and virological (RT-PCR), have been developed for the epidemiological monitoring of bovine, ovine and caprine species.
- Serological screening of all seronegative breeding animals is conducted regularly to continually monitor the spread of the virus and ascertain the individual status of the animals.
- No proof of females being infected by semen from seropositive bulls has been provided, either by natural mating or insemination.
- At least 10 million doses of semen from SBV seropositive bulls, having tested negative by RT-PCR for virus, have been exported from EU Member States to Third Countries around the world without any reports of such semen causing infection in inseminated cows.
- The epidemiological risk for unaffected third countries is more closely tied to the potential introduction of infected vectors in their regions, rather than the use of RT-PCR tested semen from seropositive bulls.

Annex: Trade Statistics

Source: Eurostat, Dataset International Trade, EU Trade since 1988 by CN8 (DS-016890), http://epp.eurostat.ec.europa.eu/newxtweb/mainxtnet.do

Data are sorted in descending order according to the volume sold in 2017.

EU-28 exports of bovine semen to Third countries (according to their SBV requirements)

Extracted on	2018.05.08 16:23:41						
FLOW	**2 - EXPORT**	INDICATORS	**SUPPLEMENTARY_QUANTITY**				
PRODUCT	**05111000 - BOVINE SEMEN**						
REPORTER	PARTNER/PERIOD	Jan.-Dec. 2012	Jan.-Dec. 2013	Jan.-Dec. 2014	Jan.-Dec. 2015	Jan.-Dec. 2016	Jan.-Dec. 2017
EU-28	**EU28_EXTRA (Third countries)**	8.859.070	9.148.331	9.633.328	10.374.781	11.434.151	10.842.492
a) Countries that accept only semen from sero-negative bulls with restrictions (with AD):							
EU-28	US UNITED STATES	513.762	451.244	943.866	847.145	1.037.561	894.610
EU-28	CN CHINA (PEOPLE'S REPUBLIC	538.584	831.270	292.021	208.000	161.536	669.370
EU-28	BR BRAZIL	639.350	649.891	915.085	417.138	487.242	505.497
EU-28	AR ARGENTINA	305.239	281.239	247.595	308.693	303.604	395.614
EU-28	CA CANADA	292.283	329.266	370.430	395.023	310.382	327.420
EU-28	ZA SOUTH AFRICA	200.041	234.327	113.381	212.926	200.189	148.660
EU-28	EG EGYPT	47.470	24.580	65.681	59.972	81.308	61.393
EU-28	UY URUGUAY	38.652	40.731	14.675	65.908	27.811	35.799
EU-28	EC ECUADOR	52.644	25.120	29.013	14.856	35.835	23.591
EU-28	AE UNITED ARAB EMIRATES	5.104	1.610	1.862	5.541	7.910	10.032
EU-28	PY PARAGUAY			2.327	2.200	5.307	3.900
EU-28	KR SOUTH KOREA		10.000				50
EU-28	IN INDIA	37.090	270.547	50.771	124.351	3.200	
	Sub-Total	2.670.219	3.149.825	3.046.707	2.661.753	2.661.885	3.075.936

b) Countries that accept semen from sero-negative and sero-positive bulls with restrictions (with AD):							
EU-28	TR TURKEY	2.484.414	2.363.637	2.322.683	3.324.545	4.016.613	2.760.271
EU-28	MA MOROCCO	307.136	406.783	252.131	197.253	338.963	378.818
EU-28	AU AUSTRALIA	245.572	209.995	236.045	206.687	221.219	325.542
EU-28	RU RUSSIAN FEDERATION (RUS	111.625	296.609	96.586	264.565	367.771	247.145
EU-28	MX MEXICO	358.420	338.742	291.842	325.024	285.866	237.882
EU-28	PE PERU	21.660	5.730	5.113	10.295	33.650	224.587
EU-28	XS SERBIA	188.190	131.207	216.857	184.209	151.757	176.418
EU-28	CO COLOMBIA	126.495	119.662	138.279	98.105	78.749	102.257
EU-28	UA UKRAINE			43.126	54.460	77.652	73.017
EU-28	JP JAPAN	51.903	98.718	165.154	89.580	116.889	53.892
EU-28	CR COSTA RICA	13.554	20.425	23.111	24.255	24.539	25.539
EU-28	LK SRI LANKA (ex CEYLAN)	3.080	2.630	1.500	6.000	15.000	5.390
EU-28	OM OMAN						3
	Sub-Total	3.912.049	3.994.138	3.792.427	4.784.978	5.728.668	4.610.761

Trade statistics (cont.):

		Jan.-Dec. 2012	Jan.-Dec. 2013	Jan.-Dec. 2014	Jan.-Dec. 2015	Jan.-Dec. 2016	Jan.-Dec. 2017
c) other countries:							
EU-28	VN VIETNAM (excl. NORTH -> 1	93.264	266.208	426.817	524.687	693.044	493.917
EU-28	CL CHILE	271.328	212.524	265.880	294.006	219.264	355.559
EU-28	CH SWITZERLAND (incl. LI->199	279.297	307.518	322.795	330.899	340.009	276.124
EU-28	BA BOSNIA AND HERZEGOVINA	139.455	195.349	287.319	235.996	138.996	242.803
EU-28	TN TUNISIA	464.101	112.949	374.844	240.543	407.722	234.601
EU-28	KE KENYA	141.448	142.248	181.804	194.461	255.156	199.423
EU-28	AL ALBANIA	196.067	132.198	152.707	139.694	138.016	183.037
EU-28	IR IRAN, ISLAMIC REPUBLIC OF	85.937	131.958	96.071	84.701	136.271	172.568
EU-28	PK PAKISTAN	71.065	77.551	52.575	95.578	78.428	145.025
EU-28	MK FORMER YUGOSLAV REPUB		18.688	26.756	53.176	40.191	141.809
EU-28	NZ NEW ZEALAND	67.111	62.815	88.750	66.636	41.033	79.194
EU-28	TH THAILAND	8.775	8.400	9.895	33.740	36.600	70.957
EU-28	BY BELARUS (BELORUSSIA)	2			38.310	61.464	60.365
EU-28	XK KOSOVO	63.237	15.401	53.320	58.248	27.200	60.300
EU-28	RW RWANDA		5.000	6.500	5.000	10.000	57.930
EU-28	ME MONTENEGRO			4.310	95.690	41.475	55.776
EU-28	UZ UZBEKISTAN		15.000	15.500	10.000	11.200	55.032
EU-28	IL ISRAEL (GAZA and JERICHO->	20.445	16.765	8.612	18.330	19.122	43.820
EU-28	MY MALAYSIA	30.140	8.120	17.517	17.528	36.915	35.024
EU-28	KZ KAZAKHSTAN	2			30.000	500	22.875
EU-28	NO NORWAY (incl.SJ excl.1995,	58.784	46.459	62.556	63.263	62.958	22.169
EU-28	IQ IRAQ	71	175	10		43.252	22.000
EU-28	BI BURUNDI	21.540	500	10.002	54.000	600	9.790
EU-28	AF AFGHANISTAN	13.000		8.200		12.746	8.937
EU-28	AZ AZERBAIJAN	21.814	26.166	18.000	8.213	15.201	7.600
EU-28	ZW ZIMBABWE (RHODESIA ->19	3.600	4.767	2.450	8.262	7.400	6.750
EU-28	BF BURKINA FASO (UPPER VOL	7.100	940	210	3.500	11.631	6.738
EU-28	LB LEBANON	32.451	13.710	19.601	17.221	15.036	6.387
EU-28	ML MALI	1.975	1.351	3.389	325	200	6.300
EU-28	MD MOLDOVA, REPUBLIC OF		4.120	11.500		10.350	6.221
EU-28	TJ TAJIKISTAN	5.550	4.000		2.350	2.000	6.200
EU-28	SA SAUDI ARABIA	14.882	900	30.124	8.260	2.355	5.040
EU-28	KG KYRGYZ, REPUBLIC (ex KYRG	6.200				1.902	5.000
EU-28	HN HONDURAS	1	20			2.000	4.947
EU-28	QA QATAR			3.000	10.020	1.550	4.503
EU-28	SD SUDAN		1.200		2.650		4.500
EU-28	NG NIGERIA	320	1	1.610	2.050	1.723	3.999
EU-28	AM ARMENIA				2.745	500	3.400
EU-28	BT BHUTAN		1.300	4.230		6.100	3.300
EU-28	SV EL SALVADOR		1.779			3.370	2.910
EU-28	BW BOTSWANA	6.240			100		2.895
EU-28	MR MAURITANIA (incl.Sp SAH.1	1.110	3.700	6.600	2.000		2.224
EU-28	GT GUATEMALA		2.200	775		2.148	2.050
EU-28	SN SENEGAL	35.510	24.411		17.275	3.004	1.607
EU-28	TM TURKMENISTAN				5.400	4.999	1.501
EU-28	LY LIBYAN ARAB JAMAHIRIYA (L				1.032		1.500
EU-28	FO FAROE ISLANDS	691	1.000	920	1.574	800	1.462
EU-28	ER ERITREA				1		1.260
EU-28	KW KUWAIT	180		1.561		516	1.051
EU-28	TD CHAD	905	875	450	450	200	1.050
EU-28	MW MALAWI						1.000
EU-28	TZ TANZANIA, UNITED REPUBLI	1.426	3.840	6.001	4.700	394	1.000
EU-28	other Third countries	111.028	130.832	174.860	145.436	96.961	3.895
	Sub-Total	2.276.052	2.002.938	2.758.021	2.928.050	3.042.502	3.155.325

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Appendix:

European inter-laboratory comparison of Schmallenberg virus (SBV) real-time RT-PCR detection in experimental and field samples: The method of extraction is critical for SBV RNA detection in semen

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Abstract:

Molecular methods for the detection of Schmallenberg virus (SBV) RNA were rapidly developed after the emergence of this novel orthobunyavirus in Europe. The SBV epizootic wave has declined, but infectious SBV in SBV RNA–positive semen remains a possible risk for the distribution of SBV. However, the abilities of SBV molecular detection methods used at European laboratories have not yet been assessed, to our knowledge. The performances of extraction and real-time reverse transcription polymerase chain reaction (RT-qPCR) methods used at 27 German and 17 other European laboratories for SBV RNA detection in the matrices of whole blood, serum, tissue homogenate, RNA eluates, and bovine semen were evaluated in 2 inter-laboratory trials with special emphasis on semen extraction methods. For reliable detection of viral genome in bovine semen samples, highly effective extraction methods are essential to cope with the potential inhibitory effects of semen components on PCR results. All methods used by the 44 laboratories were sufficiently robust to detect SBV RNA with high diagnostic sensitivity (100%) and specificity (95.8%) in all matrices, except semen. The trials demonstrated that the published recommended semen extraction methods (Hoffmann et al. 2013) and a combination of TRIzol LS, with an alternative extraction kit, have a considerably higher diagnostic sensitivity to detect SBV RNA in semen up to a detection limit of Cq ≤35 compared to other extraction methods used. A thorough validation of extraction methods with standardized semen batches is essential before their use for SBV RNA detection in bovine semen.

Key words: Detection; extraction; polymerase chain reaction; ring test; Schmallenberg virus; semen.

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