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Schmallenberg virus infection in South American camelids: Field and experimental investigations

Claudia Schulz*, Martin Beer, Bernd Hoffmann

Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Südufer 10, 17493 Greifswald-Insel Riems, Germany

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ABSTRACT

During the first epizootic wave of the novel, teratogenic Schmallenberg virus (SBV, *Orthobunyavirus*) in ruminants in Northern Europe, serological evidence of a previous SBV-infection demonstrated that South American camelids (SAC) are also susceptible to SBV. However, their potential role in SBV spread remains unknown. To investigate the prevalence and course of SBV-infection in SAC, a German field study and an animal trial with three llamas and three alpacas were conducted.

From September 2012 to December 2013, 313 of 502 SAC (62.35%) were found SBV seropositive, but negative for SBV-RNA. The estimated between-district (94.23% of 52) and median within-district (71.43%) and herd (73.13%) SBV seroprevalence in German SAC was similar to the seroprevalence reported in cattle herds and sheep flocks at the time. An age of >1 year was found a statistically significant risk factor for SBV-infection, which could be explained by the spatio-temporal spread of SBV in Germany during the study period. No clinical signs or an increase of abortion and congenital malformation associated with SBV-infection in SAC were reported by the study participants.

Similar to SBV-infected ruminants, SBV-RNAemia in experimentally SBV-infected SAC was detected for a short time between days 3 and 7 after infection (dpi), and seroconversion occurred between 9 and 21 dpi.

Despite the similar virological and serological results, the lack of clinical signs and congenital malformation associated with SBV-infection suggests that SBV causes subclinical infection in SAC. However, their role as reservoirs in the spread of SBV has to be further investigated.

1. Introduction

Since the emergence of the novel Schmallenberg virus (SBV) in North-Western Europe in 2011 (Hoffmann et al., 2012a), this *Culicoides*-borne orthobunyavirus has rapidly spread throughout Europe (Gubbins et al., 2014; Wernike et al., 2014). After the first epizootic wave 2011/12, SBV-spread slowed down considerably in Germany and neighbouring countries during 2012/13. However in 2014, acute SBV infection was confirmed in a marked number of ruminants in Germany and the Netherlands. The current extent of SBV within and outside Europe is unknown, because SBV infection is not notifiable to the World Organisation for Animal Health (OIE) (Beer et al., 2013; Conraths et al., 2013; Méroc et al., 2013b; ProMED-mail, 2014; Veldhuis et al., 2015; Wernike et al., 2015).

Similar to closely related Orthobunyaviruses belonging to the Simbu serogroup such as Akabane virus (AKAV), clinical signs in SBV-infected adult domestic ruminants are mild or inapparent and may include hyperthermia, diarrhoea and a reduction in milk yield and fertility parameters (Veldhuis et al., 2014b). Transplacental transmission of SBV in the first third of pregnancy may cause abortion and congenital malformation such as distortion and deformation of limbs, vertebra and central nervous system summarized as the arthrogryposis-hydranencephaly syndrome (AHS) (Garigliany et al., 2012b; Wernike et al., 2014). Furthermore, venereal transmission by semen contaminated with infectious SBV might contribute to its spread to SBV-free regions (Schulz et al., 2014). The main economic impact of SBV has been caused by international trade restrictions imposed on live ruminants and their products (semen and embryos) from SBV-affected regions, while the financial losses have been limited on individual SBV-affected farms (Beer et al., 2013; Veldhuis et al., 2014b).

Clinical signs and fatality in a few South American camelids (SAC) naturally infected with the Phlebovirus Rift Valley fever virus in South Africa in 2010 demonstrated their susceptibility to a member of the *Bunyaviridae* family (ProMED-mail, 2011). In Old World camelids (OWC), antibodies against the closely related AKAV

* Corresponding author.
E-mail address: claudia.schulz@fli.bund.de (C. Schulz).

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were found in 51–70% of OWC tested in Kenya, Australia and on the Arabian Peninsula (Al-Busaidy et al., 1988; Cybinski et al., 1978; Glyn Davies and Jessett, 1985), while no antibodies were detected against Peaton and Aino virus in Australian field studies (Cybinski and St George, 1978; St George et al., 1980). Recently, serological evidence of a previous SBV-infection demonstrated for the first time that SAC and OWC are also susceptible to SBV (Jack et al., 2012; Schirrmeier et al., 2012).

To date, the SBV pathogenesis in SAC and their role in the epidemiology of SBV are unknown. In Germany, SAC have become increasingly popular. The German population is currently estimated between 7,000 and 15,000 animals (Gauly, 2011b; Schulz, 2012). Accordingly, a German field study and an animal trial were conducted to gain insight into the progression of SBV infection and disease in SAC and their offspring ('cria'). Reference material was collected from experimentally infected SAC to validate serological and virological assays for SBV diagnosis in SAC.

2. Material and methods

2.1. German field study of SBV infection in SAC

To investigate the incidence and clinical outcome of SBV infection in SAC, a field study was carried out in Germany from September 2012 to December 2013. A total of approximately 250 SAC owners, veterinary laboratories and universities in Germany were contacted by email and asked to submit blood and post-mortem samples of diseased or healthy SAC and to complete a provided questionnaire. Information was requested on individual and on herd level and included sampling date, location, type of sample material (serum or whole-blood collected before or after colostrum intake, type of organ or swab material), age (newborn, <1, 1–2 and >2 years of age), sex, species and an animal identifier. Additional questions included observed clinical signs (fever, diarrhoea), disorders associated with pregnancy and birth (abortion, preterm delivery or stillbirth) and congenital malformations (ankylosis, distortion or deformation of limbs, neck or jaw, blindness and disorientation) similar to AHS as described for SBV-infected domestic ruminants and their offspring, respectively (Conraths et al., 2012; Garigliany et al., 2012b; Hoffmann et al., 2012).

2.2. Experimental infection of SAC with SBV

Three llamas (*lama glama*) (L1–L3) and three alpacas (*Vicugna pacos*) (A4–A6) were purchased in Germany and transferred to the high containment facility of the FLI, Isle of Riems, in January 2013. The animals were aged between 6 and 8 months (L1, L2, L3, A6) and 3 to 4 years (A4, A5). After several days of acclimatisation each animal was subcutaneously infected with 1 ml of SBV-containing bovine serum (1.3×10^3 50% tissue culture infectious dose per mL) well characterised by previous animal trials (Wernike et al., 2012; 2013a; 2013b).

The six SAC were monitored daily for clinical signs and rectal body temperature. Serum and whole-blood were collected at −1, 2, 3, 4, 5, 6, 7, 9, 11, 14, 17, 21, 28, 40, 47 and 54 dpi. Euthanasia of all SAC was conducted at 62 dpi as described previously (Schulz et al., 2012b). Post-mortem samples included spleen, lung, liver, tonsil, cerebrum, cerebellum, medulla oblongata as well as mandibular, mesenteric and mediastinal lymph nodes.

2.3. Virological analyses

Viral RNA was extracted from serum and whole-blood obtained during the field study and from experimentally infected SAC using QIAamp® Viral RNA Mini kit (Qiagen, Hilden, Germany) or MagAttract® Viral RNA Mini M48 kit at a KingFisher® Flex workstation (Thermo Fisher Scientific, Schwerte, Germany) according to the manufacturers' instructions. Of the post-mortem samples, pea-sized pieces were homogenised in 500 µl of medium with TissueLyser (Qiagen) using steel beads and were subsequently centrifuged at maximum speed in a microcentrifuge (≈15,000 × *g*).

Table 1
Within-herd seroprevalence of herds with at least 10 tested SAC by herd. Seropositive animals were found in all herds. In the herds with seropositive crias, the presence of maternal SBV antibodies cannot be excluded.

Region of Germany	Federal state	Herd-ID	SAC >1 pos/tested (%)	Cria pos/tested (%)	Total no. pos/tested (%)[a]	(95% CI)	% Positive by federal state[b]
West	North Rhine-Westphalia	5	9/11 (81.9)	–	10/12 (83.33)	(51.59–97.91)	32.56
	Rhineland-Palatinate	12	11/15 (73.3)	0/4 (0.0)	11/19 (57.89)	(33.50–79.75)	71.79
East	Saxony	47	14/22 (63.6)	1/2 (50.0)	15/24 (62.50)	(40.59–81.20)	70.59
		51	9/10 (90.0)	–	9/10 (90.00)	(55.50–99.75)	
Central	Hesse	3	20/30 (66.7)	3/14 (21.4)	23/44 (52.27)	(36.69–67.54)	69.44
		9	17/22 (77.3)	0/1 (0.0)	17/23 (73.91)	(51.59–89.77)	
		10	16/19 (84.2)	1/1 (100.0)	18/21 (85.71)	(63.66–96.95)	
		11	12/13 (92.3)	–	17/20 (85.00)	(62.11–96.79)	
South	Bavaria	24	29/39 (74.4)	5/8 (62.5)	34/47 (72.34)	(57.36–84.38)	73.61
	Baden-Wuerttemberg	49	9/14 (64.3)	–	9/14 (64.29)	(35.14–87.24)	58.39
Total		*n* = 10	146/195 (74.87)	10/30 (33.33)	163/234 (69.66)	(57.95–66.60)	

cria, <1 year of age; SAC >1, yearling and adult SAC >1 year; CI, confidence interval.
[a] SAC of all age groups including animals without given age.
[b] For details on SBV seroprevalence by federal state see map of Germany (Fig. 1).