First evidence of Schmallenberg virus infection in domestic ruminants in Kosovo and Albania

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Summary
Schmallenberg virus (SBV), a novel Orthobunyavirus, emerged in European domestic ruminants in 2011 causing abortions and malformations in newborns and none or mild clinical symptoms in adult animals. Here, a total of 364 bovine, ovine and caprine serum samples were collected in Kosovo and Albania between May 2014 and August 2016 and analyzed for the presence of anti-SBV antibodies. Sera were tested using an enzyme-linked immunosorbent assay (ELISA), and 48 ELISA-positive samples were subsequently analyzed by serum neutralization test (SNT). The overall percentage of ELISA positive results was 17.9%; 23.1% (53/229) was the prevalence observed in Kosovo (cattle 45.5%, sheep 19.2% and goat 6.8%), while 8.9% (12/135) was that observed in Albania (cattle 11.1%, sheep 0% and goat 20.0%). SNT confirmed the presence of neutralizing antibodies against SBV in all samples tested. This is the first study reporting SBV circulation in domestic ruminants in Kosovo and Albania, with indication that this virus has been present in Kosovo and Albania at least since 2014 without being detected.

Introduction

In November 2011, a novel infectious agent named Schmallenberg virus (SBV) was reported for the first time in adult dairy cows from farms located in Northwest Germany and the Eastern region of the Netherlands (Hoffmann *et al.* 2012). SBV is a member of the genus *Orthobunyavirus* within the family *Peribunyaviridae*. Since its emergence, circulation of SBV has been confirmed from continental Europe to Scandinavia and the British Isles. From the initially most affected countries the following seroprevalences were reported: Netherlands 72.5% in cattle (Elbers *et al.* 2012), Belgium 90.8% in cattle (Garigliany *et al.* 2012a), in France 90% in cattle and 30% in goats (Gache *et al.* 2014), and in Germany 61% in cattle, 24.7% in sheep and 26.4% in goats (Wernike *et al.* 2014). Clinical signs of SBV infection include reduced milk yield, inappetence, and diarrhea in adult ruminants lasting for a few days (Hoffmann *et al.* 2012), and multiple malformations in newborns or aborted ruminants when dams are infected during a critical phase of gestation (Peperkamp 2015). The main fetal malformations induced by SBV are arthrogryposis, torticollis, ankylosis, kyphosis, scoliosis, brachygnathia inferior, hydrocephalus, and various malformations of the central nervous system, including porencephaly and hydranencephaly (van den Brom *et al.* 2012, Garigliany *et al.* 2012b, Bilk *et al.* 2012, Herder *et al.* 2012). The incidence of malformation on farms where SBV infection was confirmed varies from 2% in goat kids and 0.5%-3% in calves (Veldhuis *et al.* 2014, Dominguez *et al.* 2014) to 3%-19% in lambs (Harris *et al.* 2014, Meloni *et al.* 2017). SBV is transmitted by hematophagous biting midges (*Culicoides*) (De Regge *et al.* 2012). The presence of species of *Culicoides* of the Obsoletus and Pulicaris Complexes are reported in Kosovo (Berisha *et al.* 2010). Direct horizontal virus transmission in sheep and cattle by contact seems highly unlikely (Wernike *et al.* 2014); however there is evidence for the presence of SBV in bull semen (Ponsart *et al.* 2014, Schulz *et al.* 2014). SBV infections can be detected by molecular methods or by serology (Beer *et al.* 2013, De Regge *et al.* 2013, Wernike *et al.* 2016). The aim of this study was to investigate

the exposure to SBV of cattle, sheep, and goats in Kosovo and Albania.

Materials and methods

Serum samples were collected during the years 2014 (March, May, July, August, September, December), 2015 (February, July, September), and in 2016 (August) and were stored at - 20 °C until use. The animals originated from 102 different ruminant farms (36 cattle, 63 sheep, and 3 goats) located in 16 different municipalities in Kosovo and 6 municipalities in Albania. Serum samples were collected from a total of 364 animals including 154 cattle, 161 sheep and 49 goats. Animals were from different breeds of cattle (Simmental, Holstein, Busha and mixed breed), sheep (Bardhoka, Sharri sheep) and goat (mixed breed), and their age ranged from 1 to 10 years. Abortions were reported at least in two dairy cattle farms in Kosovo.

All sera were tested using a commercially available enzyme-linked immunosorbent assay (ID Screen® Schmallenberg virus competition multispecies, ID.vet, Montpellier, France) according to the manufacturer's instructions. As a confirmatory test, 48 ELISA-positive sera were additionally analyzed by a serum neutralization test (SNT) performed as described previously (Wernike *et al.* 2013a). All samples were tested in triplicate and the neutralizing titers were calculated as the reciprocal of the serum dilution that still inhibited > 50% of cytopathogenic effect (ND_{50}) according to Behrens and Kaerber.

Results

Antibodies against SBV were detected by ELISA in 17.9% (65/364) of the samples collected from domestic ruminants. Since the nucleocapsid protein based ELISA reacts not only with anti-SBV antibodies, but also with antibodies against closely related viruses from the Simbu serogroup (cross-reactivity) (Bréard *et al.* 2013), a subset of 48 samples that scored positive in the ELISA was additionally analyzed by the more specific SNT. All samples tested positive, the neutralizing titers against SBV ranged from 1/6 to 1/572.

The percentage of ELISA positive results in samples from Kosovo was 23.1% (53 of 229 animals) and 8.9% (12 out of 135) in Albania scored positive (8.9%) indicating the recent circulation of SBV in both countries. The highest percentage of seropositive results was found in cattle 36/154 (23.4%), followed by sheep 25/161 (15.5%) and then goats 4/49 (8.2%).The total number of seropositive farms was 40/102 (39.22%) and 14 of the 25 municipalities (56%) were affected, their geographical location is depicted in Figure 1. The individual numbers of SBV antibody positive samples per municipality and animals species are given in Table I.

Discussion

Sampled dairy cattle farms with reported abortions tested positive for SBV antibodies, therefore, future

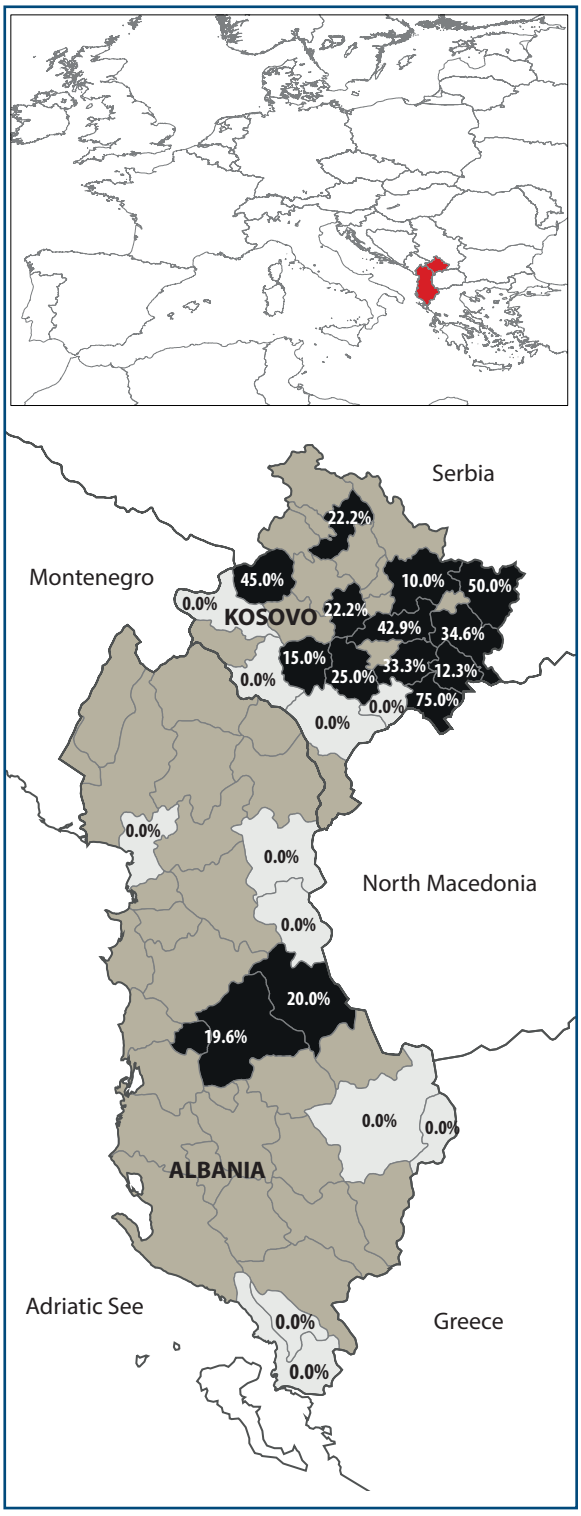

Figure 1. *Geographical location of SBV in Kosovo and Albania.*

Table I. *SBV seroprevalence in domestic ruminants in Kosovo and Albania.*

SBV seroprevalence: sample no. (positive samples)					
Municipalities	**Sample no.**	**Cattle**	**Sheep**	**Goat**	**%**
Drenas (KOS)	9	-	9(2)	-	22.22%
Ferizaj (KOS)	3	-	3(1)	-	33.33%
Gjakovë (KOS)	1	-	1(0)	-	0.00%
Gjilan (KOS)	26	2(2)	24(7)	-	34.62%
Istog (KOS)	20	19(9)	1(0)	-	45.00%
Kaçanik (KOS)	4	2(2)	2(1)	-	75.00%
Kamenicë (KOS)	14	10(7)	4(0)	-	50.00%
Lipjan (KOS)	7	-	7(3)	-	42.86%
Mitrovicë (KOS)	9	7(1)	2(1)	-	22.22%
Pejë (KOS)	2	-	2(0)	-	0.00%
Prishtinë (KOS)	20	12(2)	8(0)	-	10.00%
Prizren (KOS)	4	-	4(0)	-	0.00%
Rahovec (KOS)	40	-	40(6)	-	15.00%
Shtërpc (KOS)	1	-	1(0)	-	0.00%
Suharekë (KOS)	4	-	4(1)	-	25.00%
Viti (KOS)	65	3(2)	18(3)	44(3)	12.31%
Elbasan (ALB)	56	49(11)	7(0)	-	19.64%
Dibër (ALB)	11	11(0)	-	-	0.00%
Korçë (ALB)	14	14(0)	-	-	0.00%
Lezhë (ALB)	48	24(0)	24(0)	-	0.00%
Librazhd (ALB)	5	-	-	5(1)	20.00%
Sarandë (ALB)	1	1(0)	-	-	0.00%
Total	**364**	**154(36)**	**161(25)**	**49(4)**	**17.9%**

studies should focus on the detection of the virus itself in addition to antibodies against it. However, the viremia in adult animals is very short-lived. It lasts 3 to 6 days only (Hoffmann *et al.* 2012, Wernike *et al.* 2013b) making serological analysis a more appropriate tool for detecting SBV infection. In addition, not every malformed fetus suspected of SBV infection tested positive by PCR (De Regge *et al.* 2013, van Maanen *et al.* 2012).

The overall percentage of seropositive results in the three sampled years were: 24.75% (25/101) in 2014, 17.76% (19/107) in 2015, and 13.46% (21/156) in 2016. This apparent decrease in percentage of seropositive results has been previously observed in other affected countries as well (Meroc *et al.* 2013, Wernike *et al.* 2015). It might suggest that the virus was firstly introduced into Albania and Kosovo in 2014 or earlier and in the following years it has circulated only to a limited extent. The reduced virus circulation in 2015 and 2016 could have led to a decline in herd seroprevalence caused by a missing infection of the young livestock.

The differing percentage of seropositive results in the tested animal species, i.e. 23.4 % in cattle, 11.5% in sheep and 8.2% in goats, and their range between the municipalities are consistent with studies performed in other European countries such as the Netherlands, Belgium, France, Germany, Turkey or Greece in which SBV has already circulated. Ranges of 8.0% to 95% have been reported in cattle, 1.6% to 89% in sheep and 2.0% to 50.8% in goats (Elbers *et al.* 2012, Garigliany *et al.* 2012a, Veldhuis *et al.* 2013, Azkur *et al.* 2013, Chaintoutis *et al.* 2014, Wernike *et al.* 2014). This observation and the likewise wide range of percentages of seropositive results in the farms included in the present study could be related to the age of the animals, the landscape (availability of breeding sites of the insect vectors) or to the husbandry system as e.g. grazing has been identified as a risk factor for SBV infections when compared to herds in which animals are kept indoors (Veldhuis *et al.* 2014). The reasons for the spatio-temporal distribution of SBV seropositivity in Albania and Kosovo as well as the impact on animal production in these two countries should be evaluated in future studies.

Conclusions

This is the first report on the presence of SBV in Kosovo and Albania. Our results indicate that SBV has been circulating in domestic ruminants in Kosovo and Albania at least from the year 2014 without being detected. Further introductions of vector-borne diseases have to be expected in these two countries, therefore strengthening of the regional surveillance and control strategies for emerging pathogens in animals as a part of global scientific efforts is required.

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