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Seroprevalence of Schmallenberg virus and other Simbu group viruses among the Lebanese sheep

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Abstract

In order to evaluate for the first time, the serological prevalence of Schmallenberg virus (SBV) and other Simbu group viruses in Lebanon, sheep originating from 15 Lebanese regions were sampled in September 2016. A total number of 750 serum samples from Awassi sheep were tested by ELISA for viral nucleoprotein antibodies. From the sampled animals, 122 animals were seropositive to SBV/Simbu group viruses. The seropositive sheep were mainly located in South Lebanon. At herd-level, a seroprevalence of 53.33% was recorded in the Seven Lebanese governorates. The animal-level seroprevalence was 16.26% and both animal and herd-level seroprevalences were negative in Mount-Lebanon. Despite that there was some serological evidence showed the presence of some Simbu group viruses in the Middle East, no study was done in Lebanon. In this study, we report for the first time the prevalence of SBV and other Simbu group viruses in Lebanon.

Keywords: Lebanon, SBV, Schmallenberg virus, Sheep, Simbu group viruses.

Introduction

Schmallenberg virus (SBV) was identified in November 2011 by Friedrich Loeffler Institute (FLI, Island of Riems, Germany) following a metagenomic analysis of a pool of blood samples coming from a farm of the town of Schmallenberg (the Rhineland of North-Westphalia, Germany) (Gibbens, 2012; Hoffmann *et al.*, 2012). The percentages of nucleotide homology presented in the new genetic sequences made it possible to classify this new virus in the family of *Bunyaviridae*, genus *Orthobunyavirus*, serogroup Simbu (Tarlinton *et al.*, 2012).

The viruses group normally noncontagious, are transmitted by hematophagous arthropods, in particular of the mosquitos and culicoides (Hoffman *et al.*, 2012; Tarlinton *et al.*, 2012).

Between the month of November 2011 and mid-March 2012, the virus was highlighted in sheep, goats and cattle in Germany, the Netherlands, Belgium, the United Kingdom, France, Italy, Luxembourg and Spain (Lievaart-Peterson *et al.*, 2012, 2015). Thus constituting the first occurrence of indigenous circulation of *Orthobunyavirus* of the serogroup Simbu in Western Europe.

However, other *Orthobunyavirus* were identified in Europe (case of the Batai virus in Germany), Africa, Asia, the Middle East, and Australia (cases of Akabane virus), either sporadically by the analysis of pools of mosquitos (Jöst *et al.*, 2011; Horne and

Vanlandingham, 2014), or due to an endemic presence (case of the Tahyna virus) (Bennett *et al.*, 2011).

In fact, back in 1980 an outbreak of Akabane virus was confirmed in cattle in the Turkish Province of Aydin. Thereafter, many Middle East countries were screened for the presence of neutralizing antibodies to Akabane virus. The results showed that the virus was present in the south Turkish coast, Cyprus, the Orontes river valley in Syria and the lower Jordan River valley.

Interestingly, the fact that this virus failed to persist in southern Turkey for more than two years indicates that the Middle East may be open to epidemic rather than endemic infection.

The presence of neutralizing antibodies in the eastern Turkish Provinces of Gaziantep and Diyarbakir suggests that this might be the route whereby Akabane virus and probably other Simbu group viruses could invade the Middle East region (Taylor and Mellor, 1994).

Regarding the infection by SBV, its associated disease appears in the adult cattle by a fall of the dairy production, fever, diarrhea being able to be severe and sometimes abortions. A congenital attack is also described in lambs, calves and kids, is characterized by malformations of the arthrogryposes / hydranencéphalitis type (Lievaart-Peterson *et al.*, 2015).

Previous work conducted by the authors on brain samples collected from malformed ovine fetuses

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(submitted for post mortem examination in December 2015), detected SBV by RT-q PCR in 2 out of the 7 tested animals (protocol generously provided by Dr. Bernd Hoffmann from the Friedrich-Loeffler-Institut). This study therefore aimed to evaluate for the first time, the seroprevalence of SBV and other Simbu group viruses in Lebanon.

Materials and Methods

Country location and background

Lebanon, located at the eastern shores of the Mediterranean, with an area of 10452 Km² most of it mountainous. The length is almost three times its width and it have the Mount-Lebanon and the anti-Lebanon that are parallel to each other, they are separated by the Bekaa plain. Narrows from north to south. Lebanon has 454 Km of land boundaries and a coast line of 225 Km (Ministry of Agriculture, 2009).

Blood sampling

In September 2016, a total of 750 sheep above 12 month of age belonging to 15 large herds (> 500 sheep per herd) were sampled. Farms were chosen randomly throughout the seven Lebanese governorates: Lebanon South (4 farms), Mount Lebanon (3 farms) Lebanon North (1 farm) Nabatiyeh (2 farms), Bekaa (2 farms), Baalbeck-Hermel (2 farms), Akkar (1 farm) (Table 1).

Table 1. Number of sampled sheep per governorate and region.

Governorate	Herd / Region	Number of samples
Lebanon South	Saida	50
	Tyr	50
	Koura	50
	Jezzine	50
Mount- Lebanon	Baabda	50
	Keserwan	50
	Chouf	50
Nabatiyeh	Bent jbeil	50
	Marjeoun	50
Akkar	Akkar	50
Bekaa	Bekaa	50
	Zahle	50
Lebanon North	Zgharta	50
Baalbeck-Hermel	Baalbak	50
	Hermel	50

A number of 50 blood sample was collected from each herd, representing at least 10% of individuals in each herd. Blood samples (25ml per sample) were collected from the external jugular vein and the serum was separated into 2ml crayo-vial and preserved at -20°C until analysis.

ELISA test

In accordance with the manufacturer's instructions, all collected sera were evaluated for anti-SBV antibody using a commercially available ELISA kit (IDEXX Schmallenberg Ab Test) (IDEXX Switzerland AG Laboratories). This kit detects SBV and cross-react with other Simbu serogroups.

Briefly, all collected sera and controls were diluted 1:10 and distributed in the wells of the microtiter plate (100 µl per well). After 60 minutes of incubation in darkness at 22–24°C, all the wells were washed 3 times with 300 µl of wash solution and added 100 µl per well of the conjugate. After 60 minutes of incubation in darkness at 22–24°C, the wells were washed once again 3 times with 300 µl wash solution and added with 100 µl of TMB substrate. After 10 minutes of incubation at 22–24°C, the color reaction was stopped by adding into each well 100 µl of the Stop solution.

The plates were read with a photometer at a wavelength of 450 nm, and the optical density (OD) of the samples was analyzed in relation to the negative and the positive controls with the formula: S/P% = (sample OD-negative control OD)/(positive control OD-negative control OD). The sample is considered negative if S/P% is <30%, suspect if S/P% is between 30% and 40%, and positive if S/P% is >40%.

The true prevalence of serologically positive animals was estimated by adjusting the apparent prevalence to the sensitivity and specificity of the test as previously described (Rogan and Gladen, 1978)

Results

Schmallenberg virus or other Simbu group viruses prevalence in Lebanon

The results of the anti-SBV/Simbu group viruses antibody detection tests (Table 2) showed that 122 of the 750 tested sheep had anti-SBV/Simbu group viruses' antibodies, representing an individual prevalence of 16.26%.

Table 2: Prevalence of SBV and other Simbu viruses in Lebanon.

Item	Total	SBV + (±STDV)	Prevalence %
Sheep	750	122 (±3.53)	16.26
Herds	15	8 (±4.24)	53.33

The sheep that were tested positive were from the 8 of the 15 herds tested for SBV Simbu group viruses. The infection rate at herd level was therefore 53.33 %.

According to the results shown in Table 2, SBV/Simbu group viruses infection varied substantially between the different Lebanese governorates, with a maximum recorded in Lebanon South and Nabatiyeh, where 30% of the analyzed animal were positive, and no seropositivity was recorded in Bekaa , Baalbeck-Hermel and Mount-Lebanon (Table 3).

Table 3: Prevalence of SBV in the different Lebanese governorates.

Governorates	Number of animals	SBV +	Prevalence %
Nabatieh	100	30 (± 1.4)	30
Lebanon South	150	48 (± 2.1)	32
Lebanon North	100	32 (± 2.1)	32
Mount Lebanon	150	0 (± 1)	0
Bekaa	100	1 (± 0.5)	1
Akkar	50	11 (± 0)	22
Baalbak Hermel	100	0 (± 0)	0

A diverse geographical variation of the seropositivity was observed in the different Lebanese regions. In the southern part of the country, 80% of the tested herds contained SBV-seropositive sheep where in Mount-Lebanon (were the lowest population of sheep is located) the percentage of infected herds was 0% (Figure 1).

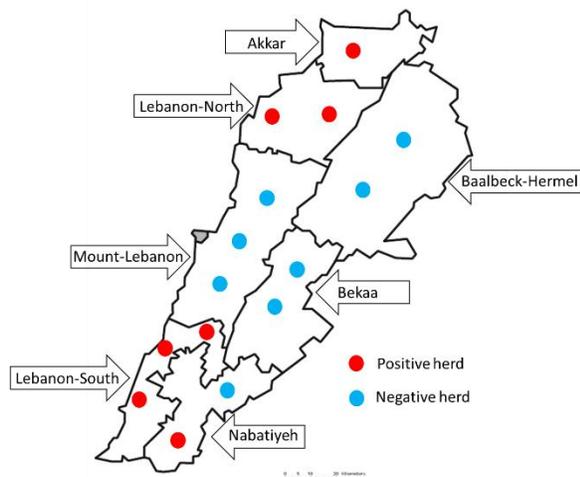


Fig. 1. Prevalence of SBV/Simbu group Viruses herds in the different Lebanese governorates.

Discussion

This is the first study that investigates the epidemiology of SBV and other Simbu group viruses' infection in Lebanon.

Herd seroprevalence in this study (53.3%) was alarming and somehow expected after the several outbreaks and sever economic losses from SBV detected from 2011 till now in many European and Mediterranean countries like Germany, France, Belgium, Italy, Spain, Greece, turkey and others.

Interestingly, our results were lower to what was previously shown in Belgium for example (above 80%

of the herds) (Méroc *et al.*, 2013) and higher than Turkey (39.8%) (Azkur *et al.*, 2013).

This study present for the first time, a clear evidence that SBV and Simbu group viruses are already spread in Lebanon and that most of the Lebanese flocks had been in contact with SBV or other Simbu group viruses somehow.

The absence of severe symptoms and any vaccination program could mean that most of the Lebanese sheep have acquired a natural protective immunity against these viruses (Sailleau *et al.*, 2013). But till today, it remains unknown how long this natural immunity could last, mainly that the Lebanese , sheep and goats are kept out on pasture day and night with only a shelter against extreme weather conditions and are not protected against parasites, mosquitos, culicoides and viruses.

For that, even in highly immunized flocks, because of all newborn animals, part of the sheep population will continuously remain susceptible.

This study should be taken into consideration as part of comprehensive SBV surveillance strategy in the country, mainly because no study is done for the detection of SBV or other Simbu group viruses and thus no prevention action are made to control the spreading of these virus.

In Lebanon, around 55% of the total population of sheep is located in the Eastern part at the Bekaa Valley. In this region, the majority of herds are large, which increase the stocking density and eventually increase the likelihood of transmission knowing that no animal in this region is yet seropositive. The absence of seropositivity in the Bekaa could be related to the absence of the vectors in this region which is characterized by rainy winters and extremely dry, and hot summers. More detailed epidemiological studies are needed to elucidate other factors, such as climate variations, presence of culicoides and rearing system that could contribute to high SBV seroprevalence and a continuous surveillance must be done in order to avoid any undesirable outbreaks.

For the moment, insufficient data are present in order to establish the origin and the time of SBV and or other Simbu viruses' infection of the sheep in Lebanon. One hypothesis is that transmission could have coincided with the importation of new animals. Yet, that there is not enough evidence to implicate the animal importation as the main source of Simbu viruses. The presence SBV already confirmed by RT-q PCR and Simbu virus-specific antibodies in most of the Lebanese herds suggests that these viruses have already spread to neighboring countries such as Syria, Palestine and Cyprus.

On the other hand, SBV coming from Greece could have been already dispersed in the area before these importations.

Even if the ELISA method used in this work does not provide a definite diagnosis for the detection of SBV, it is a recommended technique when a large number of animals needs to be tested.

Further molecular detection and phylogenetic analysis of SBV and other Simbu viruses in all the Lebanese ruminants and/or culicoides midges could reveal the source of the infections in Lebanon.

Conflict of interest

The authors declare that they have no competing interests.

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