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3 **Preliminary survey on the impact of Schmallerberg virus on sheep flocks in south of**
4 **Belgium**

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18 **Summary**

19 Between late February and May 2012, a preliminary anonym survey was conducted among
20 sheep farmers in south of Belgium in order to contribute to future estimations of the economic
21 losses caused by Schmallerberg virus (SBV). Based on clinical signs consistent with SBV
22 infection, this survey involved 13 meat sheep flocks considered as positive flocks with
23 subsequent SBV detection by RT-qPCR (PF; total of 961 animals) and 13 meat sheep flocks
24 considered as negative flocks (NF; total of 331 animals). These preliminary results indicated
25 several significant characteristics that were more present in PF than in NF. These include an
26 increased rate of abortions (6.7% in PF vs. 3.2% in NF), of lambs born at term but presenting
27 malformations (10.1% in PF vs. 2.0% in NF) and of dystocia (10.1% in PF vs. 3.4% in NF).
28 Lamb mortality during the first week of life was reported more frequently in PF (8 of 13 PF,
29 61.5%) than in NF (1 of 13 NF, 7.7%). In PF, the observed prolificacy rate was two-fold
30 lower (93%) than expected (186%).

31 The implementation of a survey at larger scale, including a high number of breeders, is
32 necessary to allow a more detailed analysis of the SBV impact in the sheep sector.

33 **Keywords:** Schmallenberg virus; Sheep; Epidemiology; Survey; Livestock impacts;
34 Economic impacts.

35 **Running title:** Impact of Schmallenberg virus on sheep flocks

36

37 **Introduction**

38 A new virus of the family *Bunyaviridae*, genus *Orthobunyavirus* has recently emerged in
39 Europe. It has been provisionally named Schmallenberg virus (SBV), following the location
40 of its first identification in Germany (Hoffmann et al. 2012). SBV was initially diagnosed by
41 RTqPCR, while serological tests have been developed more recently. The SBV is not a
42 reportable disease to the World Organization for Animal Health (*Office International des*
43 *Epizooties* - OIE). Under-reporting and under-detection are prejudicial to an accurate
44 estimation of the impact of the disease caused by SBV on livestock industry (Martinelle et al.,
45 2012). Therefore gathering farmers' estimations in matters of apparent reproductive and
46 clinical consequences of SBV infection could help to more accurately delineate the effects of
47 the disease on sheep flocks.

48

49 **Materials and methods**

50 In 2010, a total of 1,223 sheep flocks (with professional incomes) were registered in Walloon
51 Region (South of Belgium), including 48,000 animals (D GARNE, 2012).

52 Five hundred Walloon breeders are identified as members of the inter-professional federation
53 of goats and sheep in the south of Belgium (*Fédération Interprofessionnelle Caprine et Ovine*
54 *Wallonne*, FICOW). Among these, 367 members hold meat sheep flocks.

55 A solicitation to participate to an anonymous survey was sent by the Journal "*Filière ovine et*
56 *caprine*" to all members of the FICOW (Vandiest, 2012). The purpose of this survey was to
57 gather first field clinical observations, including any disorders encountered during the
58 lambing period.

59 This survey took place in south of Belgium between late February and May 2012. The time
60 period of the reported lesions corresponds to the period from May 2011 until February 2012.

61 A SBV-positive flock (PF) was defined as a flock for which at least one suspected animal
62 with clinical signs consistent with SBV infection was submitted to the laboratory with
63 subsequent positive RT-qPCR result (USDA, 2012). A SBV-negative flock (NF) was defined
64 as a flock for which no clinical signs consistent with SBV infection were observed. Attempts
65 of SBV detection were performed using the brain stem and cerebellum of the foetuses (Cay et
66 al., 2012). Recommendations issued in the note accompanying the survey explicitly specified
67 that all flocks could participate, regardless of their SBV status (PF or NF).

68 The comparison of prolificacy rates and the comparison of the number of breeding females in
69 PF and NF was realized using a paired non-parametric Wilcoxon signed-rank test and a non-
70 parametric Mann–Whitney test, respectively (the hypothesis of normality of the distributions
71 could not be verified). The frequency of clinical signs has been assessed with Pearson’s chi-
72 squared test or a Fisher’s exact test depending of conditions of use (Petrie and Watson, 2006).

73

74 **Results and discussion**

75 Responding farmers were divided in two groups, depending on the detection of SBV
76 (confirmed by RT-qPCR) in their herds: 13 SBV positive meat sheep flocks (PF; total of 961
77 animals) and 13 SBV negative meat sheep flocks (NF; total of 331 animals). In total, it
78 represents a sample of 5% (*i.e.* 26/500) of all members of the FICOW or 7% (*i.e.* 26/367) of
79 meat sheep breeders that are members of the FICOW. This rate represents the lower limit of
80 what is expected for this type of investigation (Dufour, 1994). The farmers who participated
81 in the survey are from all provinces of the Walloon Region (south of Belgium) (**Figure 1**). In
82 addition, farmers who responded reported variable levels of losses in their flocks (with first
83 report at January, 2012). In this condition the presence of bias (*i.e.* over representation of
84 severely affected flocks) had probably a limited impact.

85 The numbers and characteristics of sheep considered in this survey are listed in **Table 1**.
86 Different sheep breeds used for meat production were represented with a predominance of
87 Texel.

88 The observed and the expected prolificacy rates for each flock was estimated considering the
89 breed and aggregated by group (PF and NF) (Babo, 2000; Laignel et Benoit, 2005). No
90 difference occurred in NF between the observed and the expected values (Wilcoxon signed-
91 rank test; $P = 0.12$) but for PF, the aggregated observed prolificacy rate was significantly

92 lower (93%) than the aggregated expected prolificacy rate (186%) (Wilcoxon signed-rank
93 test; $P = 0.01$). This represents a two-fold reduction of the expected prolificacy. No significant
94 difference was observed between PF and NF in term of the starting date and duration of the
95 first lambing period. However, it appears that the number of breeding females was
96 significantly higher in PF (average of 41, median of 23, minimum of 5 and maximum of 154)
97 compared to NF (average of 11, median of 8, minimum of 2 and maximum of 26) (Mann-
98 Whitney test; $P = 0.01$) (**Figure 2**). This finding should be in accordance with the hypothesis
99 of a wide exposition of flocks to the SBV and a higher probability to detect SBV in flock with
100 an increased number of breeding females.

101 Clinical signs encountered in the two groups were compared. It appeared that the observation
102 frequency of stiff joints was significantly higher in PF (11/13) compared to NF (2/13)
103 (Fisher's exact test, $P = 0.045$). The abortion rate (Chi 2 test, $P = 0.04$) and the number of
104 stillborn or lambs dying right after being born (Chi 2 test, $P < 0.001$) were significantly higher
105 in PF compared to NF (**Table 2**). Reported Schmallenberg virus-associated lesions were
106 similar to those attributed to SBV in previous reports (Herder et al., 2012; van Maanen et al.,
107 2012).

108 The flock dystocia rate was significantly higher (mean 18.5%, median 13%, minimum 0%
109 and maximum 66.7%) in PF compared to NF (mean 6.4%, median 0%, minimum 0% and
110 maximum 83% in only one flock of small size) (Fisher's exact test, $P < 0.001$). In addition,
111 lamb mortality during the first week of life was reported more frequently by farmers in PF (8
112 of 13 PF, 61.5%) than in NF (1 of 13 NF, 7.7%) (Fisher's exact test, $P = 0.01$).

113 A symptomatic treatment (antibiotics and / or anti-inflammatory) was administered
114 occasionally after dystocia in 10 out of 13 PF. The average duration of treatment was 3.5 days
115 (minimum 2 and maximum 6 days). The mean percentage of treated animals per flock was
116 18.5% (minimum 0% and maximum 67%). The cost of treatment per animal averaged € 50.4
117 (median 50, minimum 8 and maximum € 124.5).

118 The number of lambs born at term but deformed was significantly different between PF and
119 NF (Chi 2 = 16.4; $P < 0.001$) and reached 10% in PF compared to 2% in NF. This percentage
120 is not significantly different from that observed in France and obtained with a greater number
121 of PF, *i.e.* 11.7% (Chi 2 = 1.33, $P = 0.25$) (Dominguez et al., 2012).

122 Schmallenberg virus affection does not figure among reportable diseases list (Royal Order,
123 20.11.2009); therefore, it is hard to achieve a representative view of the real situation because
124 of the risk of underreporting (Martinelle et al., 2012). Moreover, the detection by RTqPCR is
125 also limited by the short length of the viraemia, ranging from 2 to 5 days in experimentally
126 infected adult cattle (Hoffmann et al., 2012). In addition, organ distribution of SBV-RNA in
127 malformed newborns, especially in lambs, is an important component to take into account to
128 allow an increase of the sensitivity of the diagnostic strategy as demonstrated by Bilk and
129 collaborators (2012). Furthermore in a recent study, Hahn et al. (2012) found that only 12 %
130 of RT-qPCR positive calves were also positive by *in situ* hybridization, most likely because of
131 a lower sensitivity of the latter technique, unsuitable to detect SBV mRNA in tissues with low
132 SBV mRNA copy number and/or reduced viral load. In addition, in another study, Maanen et
133 al. (2012) reported that only 42 % ELISA positive fetuses were also positive by RT-qPCR.
134 This percentage was even lower for animals without malformations and provides support to
135 the superiority of ELISA as a reliable and sensitive diagnostic test. Therefore it is likely that
136 some negative flocks may have been misestimated. Consequently, the zootechnical impact of
137 SBV infection might be slightly different than these preliminary results suggest.

138 In contrast to bluetongue disease, the emerging disease caused by SBV was characterized by a
139 very large and fast geographic spreading (Beer et al., 2012). In sheep, in acute phase of the
140 disease, no particular alteration was observed in adults. This fact participates to the silent
141 spreading of the disease. This is supported by a recent EFSA report, which highlights the
142 underreporting of SBV cases (European Food Safety Authority, 2012). Retrospective
143 epidemiologic studies would bring to light useful data to clarify more accurately spatio-
144 temporal circumstances of SBV emergence in Belgium.

145 This preliminary survey allowed a better characterization of SBV-related economic losses in
146 meat sheep flocks and will allow refining questionnaires for a larger scale use.

147

148 **Conclusion**

149 Measuring the extent of the episode of SBV on livestock and zootechnical performances
150 requires further research efforts. As these results are preliminary and exploratory, an
151 implementation of the survey on a larger scale, including a larger number of farmers is needed
152 to allow a more detailed analysis of the impact of SBV in the sheep sector.

153

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157

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204 **Tables and Figures caption**

205

206 **Table 1.** Characteristics of sheep being monitored by the participating sheep farmers

Sheep category	Number of animals present in sheep flocks with		Total
	PF	NF	
Meat sheep less than one year	422	153	575
Meat sheep over one year	496	167	663
Breeding rams	43	11	54
Total	961	331	1292

207

208 Legend: PF, SBV positive flocks; NF, SBV negative flocks. Status of the flocks was assigned
209 based on RT-qPCR results.

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215 **Table 2.** Comparison of reproductive and clinical parameters

Variable	PF (N = 13)	NF (N = 13)
Number of pregnant primiparous ewes	22	26
Number pregnant multiparous ewes	505	119
Number of abortions	35 (6.7%)	8 (3.2%)
Number of clinically healthy	366 (70.0%)	216 (85.4%)
Number of stillborn or died at birth lambs	69 (13.2%)	24 (9.5%)
Number of lambs born at term but malformed	53 (10.1%)	5 (2.0%)

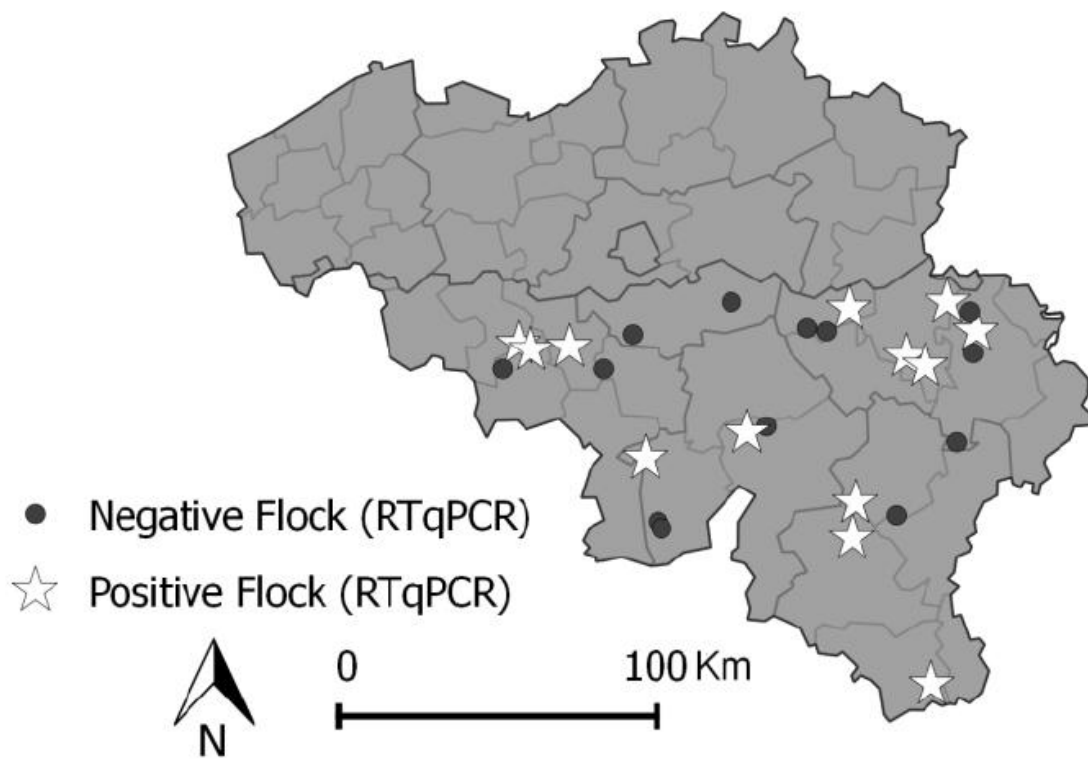
216

217 Legend: PF, SBV positive flocks; NF, SBV negative flocks. Status of the flocks was assigned
218 based on RT-qPCR results.

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221 **Figure 1.** Localization of the flocks originating from the south of Belgium included in the
222 survey in relation to their SBV status



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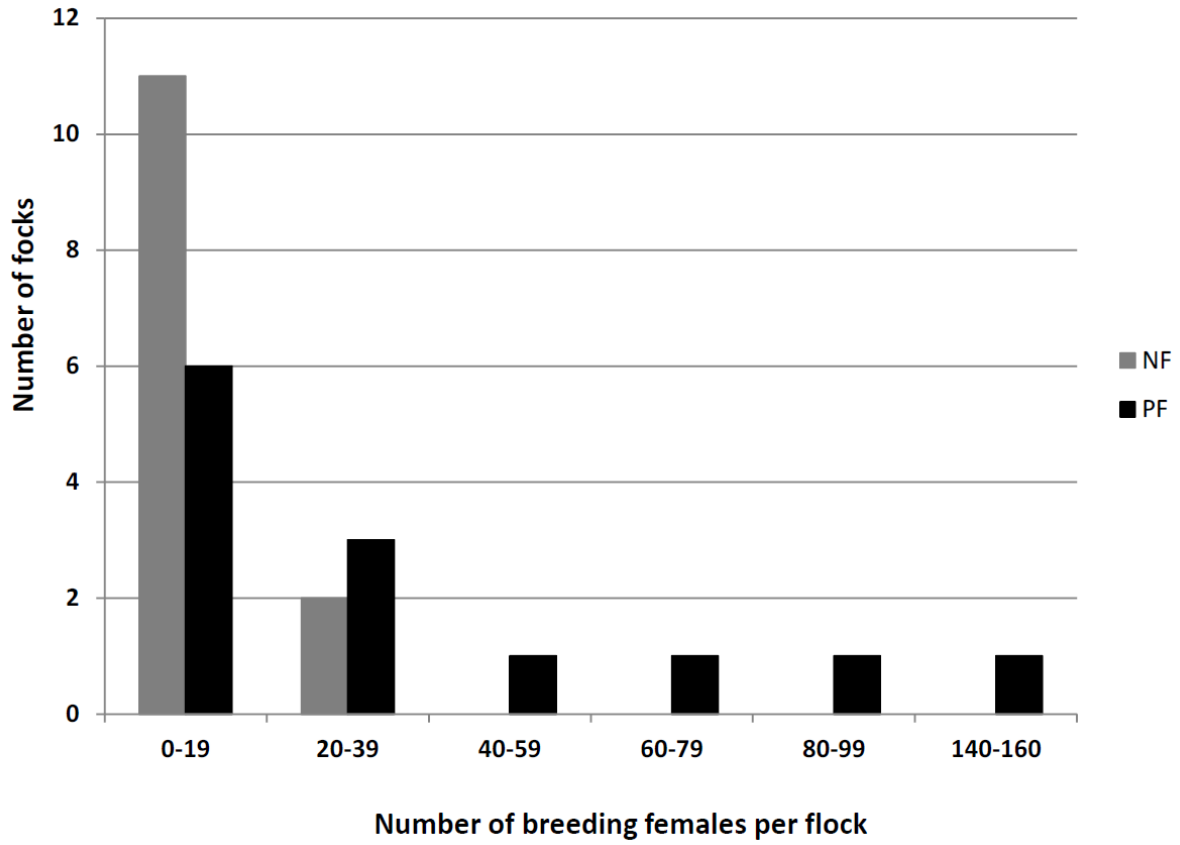
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226 **Figure 2.** Number of breeding females in function of SBV flock status

227 Legend: PF, SBV positive flocks; NF, SBV negative flocks. Status of the flocks was assessed
228 based on RTqPCR results.

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