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# Paper

## Limited interlaboratory comparison of Schmallenberg virus antibody detection in serum samples

W. H. M. van der Poel, B. Cay, S. Zientara, F. Steinbach, J. F. Valarcher, A. Bøtner, M. H. Mars, R. Hakze-van der Honing, H. Schirrneier, M. Beer

**Eight veterinary institutes in seven different countries in Europe participated in a limited interlaboratory comparison trial to evaluate laboratory performances of Schmallenberg virus (SBV) antibody detection in serum. Seven different sheep sera and three different cattle sera were circulated, and all participating institutes were asked to test these sera using SBV antibody detection assay(s) in place in their laboratories. All laboratories within the trial performed a virus neutralisation test (VNT) as well as one or two ELISAs on all samples, and swiftly detected SBV antibodies using these assays. VNT was more sensitive in detecting SBV antibodies than several of the used ELISA assays. Based on the test results, one cattle and one sheep SBV antibody-positive serum were selected to serve as reference sera, which now can be supplied to other laboratories on request.**

### Introduction

Schmallenberg virus (SBV) was discovered in November 2011, and named after the village in Germany where it was first detected in blood samples from a dairy herd. Acute SBV infections presented with fever and reduced milk yield in dairy cattle (Hoffmann and others 2012, Muskens and others 2012). From December 2011 onward, the SBV outbreak resulted in a rapid increase of malformations in newborn lambs and calves (Van den Brom and others 2012), first in the European Union and later on also in the surrounding countries (EFSA 2012). Very soon after the SBV outbreak started, veterinary institutes in the affected countries worked together on the development of diagnostic tools, including antibody detection tests which were indispensable for the needed epidemiological surveys. Laboratories first implemented virus neutralisation tests (VNT) whereas primary focus of the industry was on the development of SBV ELISAs. In order to obtain a first impression of laboratory performances in SBV antibody

testing in Europe a simple straightforward interlaboratory ring trial was organised in June 2012.

### Materials and methods Samples

Seven sheep sera were selected by the Central Veterinary Institute (CVI) in The Netherlands. Three of these sera were previously tested negative, and four of these sera were tested positive (various antibody levels) by VNT at CVI. The set included two convalescent samples of two different sheep (four specimens), obtained from a sheep infection experiment performed at CVI. The remaining three sera were obtained from adult sheep naturally infected in the field in September 2011. Three different cattle sera were selected by the Friedrich Loeffler Institut (FLI) in Germany. Two of these had previously been tested positive (different antibody levels) and one was tested negative by VNT at FLI. The cattle sera were obtained from an experimental infection experiment performed at FLI, in 2012 (Hoffmann and others 2011).

### Participating institutes

Eight veterinary institutes from seven different countries participated in the SBV antibody detection ring trial. The following institutes were involved (alphabetical order): Agency for Food, Environmental and Occupational Health & Safety (ANSES), France; Animal Health and Veterinary Laboratories Agency (AHVLA), UK; Animal Health Service (GD), The Netherlands; Central Veterinary Institute of Wageningen UR (CVI), The Netherlands; FLI, Germany; National Veterinary Institute (DTU-VET), Denmark; National Veterinary Institute (SVA), Sweden; Veterinary and Agrochemical Research Centre (VAR-CODA), Belgium.

### Assays

Totally, the eight laboratories in the trial used six different SBV antibody detection assays. Besides the VNTs, three laboratories performed an in-house ELISA and six laboratories used one or more commercially available ELISAs. The VNTs carried out were not identical in procedure. For example, in one protocol, a higher amount of infectious virus (500TCID<sub>50</sub> instead of 100TCID<sub>50</sub>) was used and one

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**TABLE 1: Detected Schmallenberg virus (SBV) specific antibody titres in seven different sheep sera (6513, 6514, 6516, 107A, 107B, 107C and 107D) and three different cattle sera (R1, R2, R3) by virus neutralisation test (VNT) performed in eight different veterinary laboratories (A-H)**

Serum	Laboratory							
	A	B	C	D	E	F	G	H
6513	64	128	96	128	64	30	128	40
6514	<4	<2	<4	<b>2</b>	<4	<5	<b>4</b>	<b>Neg</b>
6516	48	128	96	32	32	30	48	40
107A	192	512	32	64	32-64	30	512	40
107B	192	256	384	128	64	60	64	80
107C	<4	<2	<4	<b>Neg</b>	<4	<5	<4	<b>Neg</b>
107D	<4	<2	<4	<b>Neg</b>	<4	<5	<4	<b>Neg</b>
R1	≥512	512	1536	64	≥256	320	96	640
R2	192	128	128	16	32-64	60	24	80
R3	<4	<2	<4	<b>Neg</b>	<4	<5	<4	<b>Neg</b>

Results were copied as reported by the laboratory. Titres ≤4 were considered SBV antibody negative (bold fields) (for additional information see Materials and Methods)

laboratory used BHK-21 cells while all others used Vero cells. In addition there were differences in readout (different percentages of destruction/inhibition per well). (Loeffen and others 2012; Mansfield and others 2013). For two laboratories, the in-house ELISA was developed in a collaborative study (Van der Heijden and others 2013). The commercially available assays included three different products supplied by ID-VET, IDEXX or LSI, respectively. The commercially available assays were always performed according to the manufacturers' instructions. For each assay, all participants were asked to perform an antibody titration or determine S/P values (optical density (OD) sample/OD positive control) in accordance with routine procedures in the laboratory and in accordance with manufacturers' instruction as appropriate. All results were reported to the organising laboratory and copied into tables (Tables 1 and 2).

## Results

Virus neutralisation titres are listed in Table 1. Results were in agreement for all participants. All laboratories detected SBV antibodies in the same four sheep sera (6513, 6516, 107A and 107B) and did not detect SBV-specific antibodies in the remaining three sheep sera (6514, 107C and 107D). One cattle serum was tested SBV antibody negative by VNT (R3), and two (R1 and R2) were tested positive by all participating laboratories (Table 1).

ELISA titres are listed in Table 2. ELISA assays within all participating laboratories performed well. For three out of four ELISA formats there was a complete agreement between laboratories regarding the seronegative results in sheep sera as well as in bovine sera. Of two sheep samples, the result had to be designated as 'doubtful' by two

laboratories, whereas these samples were mainly scored positive by other labs (6-8 lab/test combinations) (Table 2).

If 'doubtful' ELISA results were considered positive, a higher degree of agreement between ELISAs and VNT would be reached.

## Discussion

To obtain a first impression of laboratory performances of SBV antibody testing in Europe, a simple and straightforward interlaboratory ring trial was organised in June 2012. Eight veterinary institutes in seven different countries tested a series of seven sheep serum samples and three bovine serum samples, using VNT and ELISA. VNT results of all samples were in agreement between laboratories. ELISA results were in agreement between laboratories apart from one sheep sample and one bovine sample which were reported to be 'doubtful' in four and three laboratories, respectively.

This study gives a good impression of routine SBV serology in European veterinary laboratories, and the results are promising for VNTs as well as for ELISAs. The study was not an evaluation of the sensitivities of the different assays and it did not include an evaluation of the cut-off values of the used assays. Although not one consistent protocol was applied, VNT results were in agreement between laboratories. For ELISA results there were some discrepancies, partly because certain S/P ranges have been indicated as 'doubtful' by the ELISA manufacturers. The 'doubtful' results were S/P values (OD sample/OD-positive control) <10 per cent under the cut-off value for a positive result. If all the 'doubtful' ELISA test results were to be considered antibody positive, more ELISA results would have been in agreement with VNT results. This raises the question whether the cut-off value of the involved assays could be lowered. However, a more extensive validation of the involved assays will be needed to answer this question.

This study gives an impression of the sensitivity and the specificity of SBV antibody detection assays in different laboratories, but it has to be taken into account that a very limited number of samples were tested in this trial. To assess sensitivity and specificity of ELISAs, a much larger and more varied set of samples will have to be tested. In such a study, a common VNT protocol could serve as standard. To evaluate repeatability, reproducibility and robustness of the assays, repeated testing within laboratories will be needed.

It can be concluded that SBV antibody detection in the different veterinary laboratories within European Union (EU) is reliable. Validation work of some of the used assays in this study has been published elsewhere (Breard and others 2013, Mansfield and others 2013, Van der Heijden and others 2013), and further validation of in-house as well as commercially available ELISAs in the field is on-going. SBV antibody positive sheep and bovine reference sera have been made available and can help to continue assessment of SBV antibody detection performances in laboratories in EU and countries outside EU. For such purpose, sheep serum 6513 can be supplied by CVI, and bovine serum R1 can be supplied by FLI. If needed, SBV positive sera can be accompanied with certified SBV antibody negative sera.

**TABLE 2: Schmallenberg virus (SBV) specific antibody detection ELISA S/P values in seven different sheep sera (6513, 6514, 6516, 107A, 107B, 107C and 107D) and three different cattle sera (R1, R2, R3) by ELISAs (formats α, β, γ, δ) performed in eight different veterinary laboratories (A-H)**

Serum	Laboratory (Elisa format)										
	A(δ)	B(α)	C(α)	D(α)	E(α)	F(α)	G(δ)	H(α)	D(β)	E(γ)	F(β)
6513	<b>73</b>	<b>219</b>	<b>100</b>	<b>74</b>	<b>172</b>	<b>103</b>	<b>116</b>	<b>89.6</b>	<b>55</b>	<b>81</b>	<b>65</b>
6514	7	37	26	12	23	19	11	10.9	13	-1	9
6516	<b>65</b>	<b>152</b>	<b>94</b>	<b>77</b>	<b>170</b>	<b>110</b>	<b>105</b>	<b>&gt;100</b>	<b>59</b>	<b>80</b>	<b>77</b>
107A	<b>12</b>	<b>180</b>	<b>73</b>	<b>53</b>	<b>47</b>	<b>40</b>	<b>25</b>	35.7	<b>35</b>	<b>20</b>	<b>40</b>
107B	<b>60</b>	<b>237</b>	<b>105</b>	<b>79</b>	<b>108</b>	<b>99</b>	<b>70</b>	<b>83.2</b>	<b>59</b>	<b>35</b>	<b>68</b>
107C	-1	4	1	0	-6	4	-1	0.1	-4	-2	-7
107D	-1	1	1	1	-10	6	-3	0	-4	-2	-7
R1	<b>134</b>	<b>245</b>	<b>108</b>	<b>138</b>	<b>271</b>	<b>87.6</b>	<b>158</b>	<b>&gt;100</b>	<b>88</b>	<b>145</b>	<b>104</b>
R2	<b>94</b>	<b>155</b>	<b>90</b>	<b>50</b>	<b>120</b>	<u>56</u>	<b>116</b>	<u>52.8</u>	76	67	45
R3	7	4	1	0	5	2	-3	0	-2	-3	-6

Tests were performed according manufacturers' instructions. S/P values (OD sample/OD positive control) were copied as reported by the laboratory. Values in bold were considered SBV antibody positive using the cut-off values of the manufacturers' instructions (underlined text: doubtful) (for additional information see Materials and Methods)

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