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Short communication

Seroprevalence survey for *Salmonella* Abortusovis infection in Swiss sheep flocks

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ABSTRACT

Between 1976 and 2003, no infections with *Salmonella* Abortusovis had been officially recorded in Switzerland. Since then, however, several sheep flocks were infected and suffered massive fetal losses suggesting a re-emergence of the disease. Therefore, the aim of this study was to assess the epidemiological situation of *S.* Abortusovis infection in sheep in this country. A representative serum sample collected in 2007 in the context of certifying *Brucella* freedom included sera from 578 flocks with a total of 8426 sheep from all regions in Switzerland and the Principality of Liechtenstein. Sera were tested by ELISA for the presence of antibodies specific for *S.* Abortusovis. The cantonal seroprevalence was estimated at the sheep as well as the flock-level by taking into account (a) all flocks with one or more seropositive sheep (Flock 1+) and (b) only the flocks with two or more seropositive sheep (Flock 2+).

Flocks with seropositive sheep were found throughout the country with an overall sheep-level prevalence of 1.7%. At the flock-level, overall prevalences of 16.3% and 5.0% were found for Flock 1+ and Flock 2+ definitions, respectively. Significant sheep-level clusters were located in the cantons of Bern, the Valais and Graubünden, while significant flock-level clusters (Flock 1+ and Flock 2+) were located in the canton of Graubünden only. Our results indicate that exposure of Swiss sheep flocks to *S.* Abortusovis is wide-spread.

1. Introduction

During the lambing seasons of 2003/2004 to 2007/2008, abortion storms with up to 70% fetal losses were described in several sheep flocks in the western part of Switzerland (Von Tavel et al., 2005; Belloy et al., 2009). Laboratory examination revealed an infection with *Salmonella enterica* subspecies *enterica* serovar Abortusovis (*Salmonella* Abortusovis), a disease which is notifiable in Switzerland but nevertheless has not been reported in this country for the previous 27 years (Boss et al., 1977). *S.* Abortusovis is specifically adapted to sheep, and no zoonotic potential is known. The major clinical signs visible in infected naïve flocks are abortions in the last trimester in 30–50% of pregnant ewes. In endemically infected flocks, the incidence decreases to an average of 10% and abortions are usually limited to recently purchased sheep or ewes lambing for the first time (Jack, 1968). Following an outbreak, natural immunity is generally observed (Jack, 1971; Pardon et al., 1988) and repeated abortion storms do not occur within the same flock. The observed flock-level abortion storms with high fetal losses in the Western part of Switzerland suggested

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that this was a re-emerging disease in this region, and led to questions about the situation in other regions within Switzerland. Therefore, a study was initiated to determine the seroprevalence of *S.* Abortusovis in Swiss sheep flocks using a recently developed ELISA on sera from a nation-wide representative sample of Swiss sheep flocks collected in 2007 in the context of *Brucella melitensis* freedom certification.

2. Materials and methods

2.1. Sera

Sheep serum samples were collected in the 2007 nation-wide official cross-sectional survey of the Federal Veterinary Office to document freedom from *B. melitensis* infection. The overall sample size for that survey had been calculated according to EU legislation to ensure that a flock-level prevalence of 0.2% would be detected with 90% confidence. The required number of sheep per flock in that survey was calculated to obtain a flock-level sensitivity of 99% assuming 10% within-flock prevalence and a 90% single sheep test sensitivity. The total sample size was stratified proportional to the cantonal small ruminant population. Flocks of different sizes were randomly selected and only sheep older than 12 months were included. In practice the sampling was implemented as follows: all sheep were sampled from flocks with up to 40 sheep, 40 randomly selected sheep from flocks with 40–99 sheep and 50 from flocks with over 100 sheep. That serum pool, subsequent to being tested for Brucellosis, was stored and made available for this survey. A total of 578 flocks with 8426 sheep from 25 Swiss cantons and the Principality of Liechtenstein (FL) were analysed (Table 1). Due to the availability of the samples in the serum sample bank the number of actual analysed samples deviated marginally from the official 2007 survey sample size of 583 flocks and 8800 sheep.

2.2. ELISA

A recently developed and validated indirect enzyme-linked immunosorbent assay (iELISA) was used to identify serologically positive sheep (Wirz-Dittus et al., 2010). The assay had sheep-level characteristics of 98% (95% CI 88.0–99.9) sensitivity (SE) and 100% (95% CI 92.1–100.0) specificity (SP), and detected antibodies specific for *S.* Abortusovis for at least 10 months after abortion (Wirz-Dittus et al., 2010).

2.3. Prevalence estimation

Crude seroprevalences, defined as number of sheep seropositive (above the ELISA cut-off) divided by number of sheep tested, were derived at the cantonal level as well as for defined subgroups such as flock size classes. Seroprevalence estimates of infected flocks were calculated for two flock status definitions: a flock was either defined as positive if one or more sheep were seropositive (Flock 1+) or if two or more sheep were seropositive (Flock 2+). The survey design module of the statistical package STATA v10 was used to derive data-structure adjusted sheep-level seroprevalence estimates with statistically based exact confidence limits. Wald statistics with associated *p*-values were utilized to interpret observed seroprevalence differences between comparison groups. A Bayesian model for a one (1) test multiple (n) sub-populations – structure (Branscum et al., 2004) was implemented in the freeware environment WinBugs 3.03 (http://mathstat.helsinki.fi/openbugs/) in order to derive sheep and flock-level true prevalence estimates by canton while adjusting for the imperfect ELISA test characteristics. Test characteristic priors were entered as beta distributions with parameters ($a = d + 1$; $b = n - d + 1$) based on the test validation data (SE = 43/44 = 97.7%; SP = 45/45 = 100%) (Wirz-Dittus et al., 2010). For the prevalence estimates by subpopulation, uninformative priors (beta 1,1) were entered. The model was run with 110,000 iterations, and median prevalence estimates and the 95% prediction interval (defined by lower and upper prediction limits) of the last 100,000 iterations, i.e. after a 10,000 iteration burn-in phase, were extracted. Three chains of starting values were compared to visually check whether the models converged to the same stable median values.

2.4. Spatial analysis

All flocks were spatially referenced using non-earth Swiss spatial coordinates of the centroid of the 4-digit ZIP code region of the owners address using a commercially available product (www.geopostcodes.com). The Bernoulli model for purely spatial clustering within the freeware package SaTScan v8 (www.satscan.org) was used to identify case (=test – positive sheep, Flock 1+, Flock 2+) clusters. These were defined as having significantly more observed cases in a defined circular region than to be expected from the distribution of all tested (negative and positive) sheep and flocks in the population. Detection of statistically significant ($p < 0.05$) positive (case) clusters was based on a maximum circle size of 30% of the underlying population and 999 model iterations. As outcome measures, the observed-to-expected case ratio (OER) estimates and likelihood ratio test statistics were used. Spatial distribution of Flock 1+ and Flock 2+ locations as well as those of the significant clusters was plotted on a map that contained the Swiss cantonal borders and lakes using the MapInfo GIS software package (http://www.pbinsight.com/welcome/mapinfo/).

3. Results

Of the 578 sheep flocks, 94 had at least one seropositive sheep (Flock 1+) and 29 of these two or more seropositive sheep (Flock 2+). The remaining 484 flocks were seronegative. On average, 14.6 sheep (range 1–49) per flock were tested (Table 1). Cantonal sheep-level seroprevalence estimates ranged between 0% and 9.4% (sample average 1.7%). With the exception of one canton, Bayesian test-adjusted sheep prevalence estimates were consistently slightly higher (data not shown). Only five cantons and the Principality of Liechtenstein (FL) did not have any seropositive sheep (Table 1). Cantonal flock-level prevalence estimates for Flock 1+ definition ranged between 0% and 36.4% (sam-

Table 1
2007 Bayesian seroprevalence estimates and 95% prediction intervals for *Salmonella* Abortusovis at the Swiss sheep and flock-level stratified by canton. Positive flock classification was based on two different approaches: (a) one or more ELISA positive sheep in the flock (Flock 1+) or (b) two or more ELISA positive sheep in the flock (Flock 2+).

Canton	Abbr.	Sheep	Tested sample		No sheep tested/flock			Positive sheep	Sheep prevalence			Flock 1+ pos. flocks	Flock 1+ prevalence estimates			Flock 2+ prevalence estimates		
			Flocks	Sheep	Mean	Min.	Max.		Median	LPL	UPL		Median	LPL	UPL	Median	LPL	UPL
1	ZH	20886	21	293	14	2	38	10	0.040	0.020	0.071	6	0.293	0.125	0.509	0.162	0.044	0.354
2	BE	59155	105	1168	11.1	1	40	22	0.020	0.012	0.033	16	0.145	0.076	0.228	0.027	0.003	0.075
3	LU	18965	47	400	8.5	1	40	4	0.013	0.003	0.029	4	0.083	0.015	0.193			
4	UR	8832	12	202	16.8	1	40	2	0.016	0.003	0.042	1	0.117	0.010	0.364	0.123	0.013	0.366
5	SZ	21825	27	388	14.4	2	40	6	0.019	0.007	0.038	5	0.191	0.065	0.368	0.054	0.004	0.182
6	OW	3728	8	116	14.5	5	32	3	0.036	0.010	0.083	2	0.282	0.058	0.611	0.177	0.021	0.492
7	NW	4011	6	44	7.3	5	10	0	0.024	0.002	0.093	0						
8	GL	3293	5	148	29.6	20	40	0	0.009	0.001	0.034	0						
9	ZG	4347	6	148	24.7	10	39	1	0.015	0.002	0.045	1	0.222	0.025	0.590			
10	FR	14602	35	282	8.1	1	30	4	0.018	0.005	0.042	4	0.116	0.027	0.257			
11	SO	9497	10	159	15.9	2	26	4	0.033	0.011	0.073	3	0.321	0.093	0.622	0.145	0.016	0.420
12	BS	57	1	9	9	9	9	0	0.063	0.006	0.225	0						
13	BL	7220	11	126	11.5	1	40	5	0.049	0.019	0.101	2	0.208	0.038	0.488	0.133	0.015	0.392
14	SH	3258	2	5	2.5	2	3	0	0.075	0.007	0.265	0						
15	AR	9087	15	210	14	1	37	4	0.010	0.001	0.032	3	0.217	0.056	0.461	0.098	0.009	0.307
16	AI	3765	7	74	10.6	4	33	0	0.016	0.001	0.062	0						
17	SG	40672	53	761	14.4	1	40	8	0.012	0.004	0.023	6	0.109	0.032	0.220	0.042	0.005	0.124
18	GR	56002	47	1129	24	1	40	35	0.034	0.023	0.051	16	0.342	0.211	0.491	0.198	0.099	0.329
19	AG	22996	45	548	12.2	1	40	6	0.013	0.004	0.027	4	0.087	0.016	0.200	0.051	0.006	0.145
20	TG	20503	32	432	13.5	2	43	6	0.017	0.006	0.035	4	0.128	0.032	0.279	0.045	0.003	0.155
21	TI	17776	12	213	17.8	2	40	1	0.010	0.001	0.032	1	0.116	0.009	0.359			
22	VD	17318	11	176	16	1	40	4	0.030	0.010	0.066	4	0.379	0.137	0.666			
23	VS	65297	49	1240	25.3	5	49	16	0.014	0.007	0.024	9	0.182	0.082	0.312	0.046	0.005	0.133
24	NE	2682	3	32	10.7	4	19	3	0.105	0.034	0.229	1	0.386	0.054	0.826	0.391	0.061	0.823
25[a]	GE	2198	0	0	–	–	–	–	–	–	–	0						
26	JU	5612	6	91	15.2	1	35	2	0.034	0.008	0.087	2	0.364	0.082	0.726			
27[b]	FL		2	32	16	9	23	0	0.031	0.003	0.117	0						
Total			578	8426	14.6	1	49	146				94						
New estimate for sheep-level sensitivity (SE)									0.886	0.681	0.983							
New estimate for sheep-level specificity (SP)									0.999	0.996	1.000							
Flock 1+ neg. (pooled)[c]			23		0								0.029	0.001	0.147			
Flock 2+ neg. (pooled)[c]			765													0.005	0.000	0.026
Estimate for flock-level test sensitivity (SE)													0.975	0.920	0.996	0.977	0.927	0.997
Estimate for flock-level test specificity (SP)													0.984	0.952	0.998	0.991	0.983	.996

[a] The canton of Geneva did not have any sampled flocks.
[b] The principality of Liechtenstein was included in the sample and treated like a Swiss canton.
[c] For the Bayesian analysis, all cantons without positive flocks (both definitions) were pooled into one stratum to reduce strata and increase precision in the estimates.

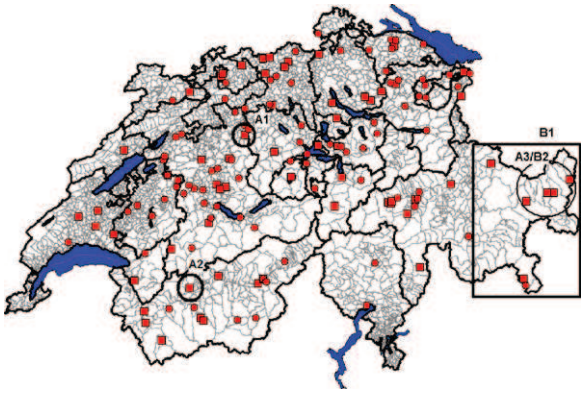

Fig. 1. Sheep flocks with a single (red dots) or two or more (red squares) positive sheep in a seroprevalence study for antibodies against *Salmonella* Abortusovis infection (2007). The underlying population of test-negative flocks is not shown. Smaller thick-lined circles (A1: OER = 50.0, $p = 0.001$; A2: OER = 13.5, $p = 0.003$) toward the west (left) represent individual flocks that were identified as significant case clusters at the sheep-level. The black rectangle with the included circle to the southeast (right) represents a statistically significant cluster of positive flocks (circle B2: threshold one positive sheep per flock (OER = 6.2, $p = .020$); square B1: threshold 2+ positive sheep per flock (OER = 10.0, $p = 0.007$). The included circle at the same time represents an individual flock (A3: OER = 6.2, $p = 0.001$) that was identified as significant case cluster at the sheep-level. Thicker regional outlines represent cantonal boundaries, and blue areas lakes. North is top.

ple average 16.3%), with some changes in both directions to be seen in the results of the Bayesian model when compared to the conventional estimates. Prevalence estimates for Flock 2+ definition ranged between 0% and 33.3% (sample average 5%). With the exception of one canton, these estimates were slightly higher in the Bayesian model approach. Compared to Flock 1+, six additional cantons were considered test-negative. Bayesian test sensitivity and specificity estimates were slightly higher with the Flock 2+ case definition (97.7%; 99.1%) when compared to those for Flock 1+ (97.5%; 98.4%)(Table 1). Observed sheep-level seroprevalence estimates did not show any significant differences between flock size categories (data not shown).

Statistically significant sheep-level spatial clusters were located in the cantons of Bern (BE), the Valais (VS) and Graubünden (GR) with the highest observed-to-expected ratio (OER = 50.0) in the canton of Bern. Statistically significant farm level spatial clusters for Flock 1+ and Flock 2+ definitions were located in the canton of GR only, with OER estimates of 6.2 and 10.0, respectively (Fig. 1).

4. Discussion

The aim of this study was to assess the seroprevalence of *S.* Abortusovis in Switzerland to determine whether the sheep population was naïve (no seroconversion). This would be consistent with the recent reports of a locally (re-)emerging disease with sporadic outbreak storms.

Although no abortions due to S. Abortusovis had been reported for several decades before 2007 the results showed that exposure to the agent (and resulting seroconversion) was endemic and that flocks with seropositive sheep could be found throughout the country, even though at a low average (across regions) prevalence of 1.7%. Since no seroprevalence studies in other countries have been published so far this result cannot be easily compared. Unexpectedly, however, was the wide-spread low-level presence of sheep with antibodies. In flocks with only a single seropositive sheep, this sheep might have been introduced into the flock after the infection and when the sheep was no longer excreting bacteria. Based on the findings of a large number of flocks with only a single reactor, the specificity of the test might not be perfect, thus resulting in some (single) reactors. In an ongoing study those flocks and positive sheep would be further investigated to clarify their infectious status. Five percent of flocks (average of 14 and maximum of 49 sheep tested per flock) had two or more test-positive sheep, suggesting a low-level but wide-spread exposure. This was unexpected given that this (notifiable) disease had rarely been reported in the past. Farmer's management practices or veterinarian's behaviour might explain this finding. They may not recognize an increase in the number/frequency of abortions, especially in small flocks and mountainous regions, and therefore not submit foetuses for laboratory diagnosis. In addition, *S.* Abortusovis infections may be missed in the laboratory. These bacteria grow much slower than other *Salmonella* serotypes, and small colonies could easily be overgrown by other bacteria. *Escherichia coli*, a frequent contaminant in aborted foetuses, may inhibit the growth of *S.* Abortusovis (Belloy et al., 2009). Finally, in Switzerland, diagnosis of ovine abortion is predominantly targeted at *B. melitensis*, *Coxiella burnetii* and *Chlamydophila abortus* (TSV Art. 129). Therefore, only analysis of cotyledons and blood are routinely done. Furthermore, the cantons pay the costs of the analysis only for staining and serology. Given that both of these methods are not available for detecting *S.* Abortusovis and chances are much lower of isolating the agent from fetal membranes than from fetal stomach contents, *S.* Abortusovis could simply be missed in the diagnosis of ovine abortions. Furthermore, as it is not known how long antibodies can be detected by the ELISA used in this study, positive sheep which had experienced an infection and seroconverted several years before sampling, could be missed resulting in an underestimation of the seroprevalence.

Highest sheep and flock-level seroprevalence estimates were found in the canton of NE and were due to the high number of positive tested sheep in one of only three tested flocks. Therefore these results are unlikely to provide a reliable estimate of the true situation in this canton but may reflect a recent *S.* Abortusovis abortion problem in the affected region (single flock). In addition, one needs to be aware that the utilized serum pool reflects the status in 2007, and that sample size was determined for the purpose of disease (threshold) detection. It therefore is too small to generate narrow confidence intervals when used for prevalence estimation. However, we consider this serum bank appropriate for assessing the spatial distribution of disease.

Sheep-level spatial clusters were identified in the cantons of BE, the VS and GR. This is in accordance with the conclusion which was drawn from identifying the numerous *S.* Abortusovis abortion cases in the canton of the VS (Von Tavel et al., 2005) and confirms the importance of this etiology for ovine abortions in that canton. There-

fore it can be assumed that there are real problems in the other two cantons with spatial clusters. Flock-level (Flock 1+ and Flock 2+) spatial clusters were only detected in the canton of GR. Together with the observed sheep-level spatial clusters and one of the highest prevalences, these results indicate that exposure to the agent (and resulting seroconversion) seems frequent in this canton. Especially the occurrence of Flock 2+ clusters indicates an active *S.* Abortusovis problem in these regions. These observations require increased disease awareness and follow-up investigations to gain more insight in the epidemiology of the disease.

Despite the high sensitivity and specificity of the ELISA used in this study, it remains important to adjust the apparent prevalence estimates for the imperfect test characteristics. We used the Bayesian model which showed relatively similar true prevalence estimates compared to apparent prevalence estimates, a consequence of the almost perfect sheep-level specificity and also high sheep-level sensitivity. At flock-level, the imperfect sheep-level sensitivity was compensated for by, on average, testing 14+ sheep per flock (and assuming that in infected flocks several sheep would be seropositive). Working with two different within-flock cut-off values allowed us to modulate the flock-level specificity and therefore, especially with the Flock 2+ threshold, yield rather low (conservative) prevalence estimates. Even then, 14 out of 26 cantons had seropositive flocks in the sample.

5. Conclusion

Although in Switzerland no cases of *S.* Abortusovis had been reported for the last few decades, this study shows that we have wide-spread serological evidence of (low-level) exposure, indicating at least a regional endemic situation comparable to what has been indicated from neighboring countries. This investigation suggests that more attention should be paid to *S.* Abortusovis in the etiology of ovine abortions in Switzerland. Therefore veterinary officials, veterinarians in practice and owners should be made aware of the problem and laboratory diagnosis of ovine abortion should include bacteriologic examination of fetal internal organs, particular abomasal contents.

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References

Belloy, L., Decrausaz, L., Boujon, P., Hächler, H., Waldvogel, A.S., 2009. Diagnosis by culture and PCR of *Salmonella* Abortusovis infection under clinical conditions in aborting sheep in Switzerland. Vet. Microbiol. 138 (3,4), 373–377.

Boss, P.H., Nicolet, J., und Margandant, A., 1977. Zum Verlauf einer *Salmonella*-abortus-ovis-Infektion in einer Schafherde. Schweiz. Arch. Tierheilkd. 119, 395–404.

Branscum, A.J., Gardner, I.A., Johnson, W.O., 2004. Bayesian modeling of animal- and herd-level prevalences. Prev. Vet. Med. 66 (1–4), 101–112.

Jack, E.J., 1968. *Salmonella abortus* ovis: an atypical *Salmonella*. Vet. Rec. 82, 558–561.

Jack, E.J., 1971. *Salmonella* abortion in sheep. Vet. Annu. 12, 57–63.

Pardon, P., Sanchis, R., Marly, J., Lantier, F., Pepin, M., Popoff, M., 1988. Salmonellose ovine due a *Salmonella* Abortusovis. Ann. Rech. Vet. 19, 221–235.

Von Tavel, L., Fivian, R., Kirchhofer, M., Boujon, P., Hirsbrunner, G., 2005. Abortion in sheep: epidemic *Salmonella* Abortusovis outbreak 2005 in Switzerland. Schweiz. Arch. Tierheilkd. 147 (10), 445–452.

Wirz-Dittus, S., Belloy, L., Doherr, M.G., Hüssy, D., Sting, R., Gabioud, P., Waldvogel, A.S., 2010. Use of an indirect enzyme-linked immunosorbent assay for detection of antibodies in sheep naturally infected with *Salmonella* Abortusovis. J. Vet. Diagn. Invest. 22 (4), 531–536.