Preventive Veterinary Medicine 206 (2022) 105697

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed

Prevalence and antimicrobial resistance of *Salmonella enterica* subsp. *diarizonae* serovar 61:k:1,5,(7) in Swiss sheep flocks

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ARTICLE INFO

Keywords:
Chronic proliferative rhinitis
Ovine
Salmonellosis
Zoonoses
Respiratory tract

ABSTRACT

Salmonella (*S.*) *enterica* subspecies *diarizonae* (IIIb) serovar 61:k:1,5,(7) (*S.* IIIb 61:k:1,5,(7)) is considered to be sheep-associated, as it can be found in the intestine, tonsils and nose of clinically healthy sheep, but it has also been described in separate clinical disorders in sheep. In particular, *S.* IIIb 61:k:1,5,(7) is described as the causative agent of chronic proliferative rhinitis (CPR) in sheep. In Switzerland, CPR in sheep due to *S.* IIIb 61:k:1,5,(7) was first described in 2017 in a flock of Texel sheep. Therefore, we assessed the prevalence of *S.* IIIb 61:k:1,5,(7) within the Swiss sheep population using a representative sampling strategy. From May 2017 to June 2018 a total of 681 nasal swabs from individual clinically healthy sheep of 141 different flocks throughout Switzerland were taken. Swabs were analysed by selective enrichment for the presence of *S.* IIIb 61:k:1,5,(7). Additionally, antimicrobial resistance of the isolates was determined by broth microdilution. A total of 146 out of 681 nasal swabs tested positive for *S.* IIIb 61:k:1,5,(7), which corresponds to a prevalence on animal level of 21% (95%CI 18%–25%). In 73 out of 141 flocks tested, at least one sheep tested positive for *S.* IIIb 61:k:1.5,(7), resulting in a minimal prevalence on flock level of 52% (95%CI 43%–60%). Positive flocks were found in all cantons except the canton of Jura. Adults were significantly more affected than sheep under one year/lambs and positive sheep were found in several breeds. No microbiologically resistant isolates were detected, except for one isolate showing resistance against ampicillin. Because of its widespread occurrence in the Swiss sheep population, further research should focus on the pathogenic impact of *S.* IIIb 61:k:1,5,(7) on the health status of sheep.

1. Introduction

Salmonella (*S.*) *enterica* subspecies *diarizonae* (IIIb) serovar 61:k:1,5,(7) (*S.* IIIb 61:k:1,5,(7)) is considered to be sheep-associated, as it can be found in the intestine and tonsils of clinically healthy sheep, but has also been described in different clinical disorders. It has been detected in the intestine of sheep without clinical signs or pathological findings in several European countries, such as Norway, Germany, Sweden, Switzerland and Spain, as well as in the US (Alvseike and Skjerve, 2002; Zweifel et al., 2004; Sörén et al., 2015; Dargatz et al., 2015; Methner and Moog, 2018; Figueras et al., 2020). Moreover, *S.* IIIb 61:k:1,5,(7) could be isolated from ovine tonsils sampled at slaughterhouses in Iceland and Switzerland (Hjartardóttir et al., 2002; Bonke et al., 2012). Recently, a high prevalence of *S.* IIIb 61:k:1,5,(7) was detected in both, nasal secretions and faeces of sheep flocks in the Spanish region of Aragon, leading the authors to assume that *S.* IIIb 61:k:1,5,(7) is endemic in Spanish sheep flocks. It is noteworthy that the isolation proportion in nasal swabs (38.5%) was higher than that in faecal samples (22.5%) (Figueras et al., 2020).

In addition to symptomless colonisation, *S.* IIIb 61:k:1,5,(7) is described in the context of clinical disorders such as abortion, enteritis or epididymo-orchitis in sheep (Davies et al., 2001; Ferreras Mdel et al.,

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https://doi.org/10.1016/j.prevetmed.2022.105697

Available online 21 June 2022

2007; Chatzopoulos et al., 2016; Schnydrig et al., 2018; Hyeon et al., 2021). Moreover, *S.* IIIb 61:k:1,5,(7) is the only *Salmonella* serovar described as the causative agent of chronic proliferative rhinitis (CPR) in sheep (Meehan et al., 1992; Lacasta et al., 2012; Stokar-Regenscheit et al., 2017). In Switzerland, chronic proliferative rhinitis in sheep was first described in 2017 in a flock of Texel sheep (Stokar-Regenscheit et al., 2017). Severe proliferation of the nasal mucosae of the turbinates in association with severe chronic inflammation was found in three deceased sheep. Consecutive analyses of the affected flock revealed a high prevalence of *S.* IIIb 61:k:1,5,(7) in nostrils (46%) but a low prevalence in faecal samples (6%) from sheep of the affected Texel sheep flock (Stokar-Regenscheit et al., 2017).

In addition to sheep, *S.* IIIb 61:k:1,5,(7) can sporadically infect humans. Recently, *S.* IIIb 61:k:1,5,(7) was isolated from the urine of a patient with a urinary tract infection (Uelze et al., 2020), underlying the zoonotic potential of this pathogen. Interestingly in this context, it was also found in ground beef in the US (Gupta et al., 2016).

Until now, in Switzerland the finding of CPR caused by *S.* IIIb 61:k:1,5,(7) and nasal colonisation was restricted to a single Texel sheep flock. Hence, the aim of this study was to determine the prevalence of *S.* IIIb 61:k:1,5,(7) in nasal swabs using a representative sample for the Swiss sheep population. Data on the spread of this pathogen within the Swiss sheep population will form the basis for evaluating of the possible need for further steps to be taken in the future.

2. Materials and methods

2.1. Sample collection

Samples were collected within the framework of a cross-sectional study for estimation of a nationwide representative prevalence of another pathogen described elsewhere (Ardüser et al., 2020). In brief, a total of 681 ovine nasal swabs were taken from May 2017 to June 2018 from 141 different flocks of sheep throughout Switzerland (Table 1). Sheep sampled, showed no clinical signs of respiratory disease, especially no sign of CRP. In all cantons, except for Uri and Basel-Stadt, flocks were randomly selected. The number of flocks per canton was calculated proportionally to their ovine population size (Table 2). The median flock size was 29 (the mean value was 47.5). Depending on the size of the flock, up to five samples were randomly collected. All sheep were tested in flocks with less than five animals. In one flock, only a single sheep was tested. The sheep were divided into three age categories, namely “adult”, “yearling” and “under one year old/lambs”, of which 494, 76 and 100 animals each were sampled (Table 3). The tested sheep belonged to 29 different breeds (Table 4). Swabs were immediately transferred into liquid Amies transportation medium (Axonlab SwabAX, liquid Amies, Axon Lab AG, Baden, Switzerland). All samples were transported to the laboratory within 48 h of collection without cooling and were cryopreserved at − 80 °C until culture.

The study was approved by the cantonal committees for animal experimentation of the cantons involved in accordance with the Swiss animal welfare legislation (approval number BE 5/17 +).

2.2. Bacteriological cultures for Salmonella spp.

In 2020, samples were thawed and subsequently enriched in Muller-Kauffmann Tetrathionate Novobiocin Broth (MKTTn, Thermo Fisher Scientific, Pratteln, Switzerland) for the detection of *Salmonella* spp. by transferring the swab into 10 mL MKTTn broth, which was then incubated at 37 °C ± 1 °C for 24 h ± 3 h. One loopful of incubated MKTTn broth was spread onto two different *Salmonella* selective agar plates (Brilliance Salmonella agar and Brilliant Green agar, Thermo Fisher Scientific), which were then incubated aerobically at 37 °C ± 1 °C for 24 h ± 3 h. Suspicious colonies on Brilliance Salmonella agar and brilliant green agar were subcultured on triple sugar iron agar plates (TSI Agar, Axon Lab AG, Baden, Switzerland) and incubated aerobically at 37 °C ± 1 °C for 24 h ± 3 h. Phenotypically suspicious strains were subcultured on trypticase soy agar with 5% sheep blood (TSA SB, Becton Dickinson AG, Basel, Switzerland) for an additional 24 h ± 3 h under the same conditions.

Table 1
Prevalence of Salmonella enterica subsp. diarizonae serovar 61:k:1,5,(7) in Swiss sheep by canton and region in 2017/2018.

Canton and Region	Number of sheep tested (n)	Number of sheep tested positive (y)	Prevalence (%)	95%CI[a]	
Bern	101	11	10.9	5.6	18.7
Fribourg	25	6	24.0	9.4	45.1
Jura	10	0	0.0	0.0	30.8
Neuchâtedl	5	2	40.0	5.3	85.3
Solothurn	19	4	21.1	6.1	45.6
Region Espace Mittelland	**160**	**23**	**14.4**	**9.3**	**20.3**
Geneva	5	3	60.0	14.7	94.7
Vaud	33	8	24.2	11.1	42.3
Valais	64	4	6.2	1.7	15.2
Ticino	29	5	17.2	5.8	35.8
Region lake Geneva & Ticino	**131**	**20**	**15.3**	**9.6**	**22.6**
Aargau	39	11	28.2	15.0	44.9
Baselland	15	1	6.7	0.2	31.9
Zurich	30	2	6.7	0.8	22.1
Region North-West Switzerland & Zurich	**84**	**14**	**16.7**	**9.4**	**26.4**
Appenzell-Innerrhoden	5	2	40.0	5.3	85.3
Appenzell-Ausserrhoden	12	4	33.3	9.9	65.1
Glarus	10	4	40.0	12.2	73.8
Graubünden	75	29	38.7	27.6	50.6
St. Gallen	69	27	39.1	27.6	51.6
Schaffhausen	5	2	40.0	5.3	85.3
Thurgau	28	3	10.7	2.3	28.2
Region Eastern Switzerland	**204**	**71**	**34.8**	**28.3**	**41.8**
Luzern	35	5	14.3	4.8	30.3
Nidwalden	10	2	20.0	2.5	55.6
Obwalden	10	5	50.0	18.7	81.3
Schwyz	32	5	15.6	5.3	32.8
Zug	15	1	6.7	0.2	31.9
Region Central Switzerland	**102**	**18**	**17.6**	**10.8**	**26.4**
Total	**681**	**146**	**21.4**	**18.4**	**24.7**

[a] Lower CI and Upper CI indicate 95% Clopper-Pearson confidence intervals

2.3. Identification and serotyping of Salmonella spp.

Salmonella spp. was determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF MS) using the direct transfer method according to the manufacturer’s instructions (Biotyper 3.0, Bruker Daltonics GmbH, Bremen, Germany). *Salmonella* spp. was identified to the species and subspecies levels using routine biochemical methods; the serovar was assigned by serotyping according to the International Organization for Standardization (ISO) 6579-3:2014 : Horizontal method for the detection, enumeration and serotyping of Salmonella − Part 3: Guidelines for serotyping of *Salmonella* spp.

2.4. Antimicrobial susceptibility testing

Minimal inhibitory concentrations (MICs) were determined by broth microdilution based on the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2018) against the following antimicrobials: ampicillin (AMP), azithromycin (AZI), cefotaxime (FOT), ceftazidime

Table 2
Prevalence of *Salmonella enterica* subsp. *diarizonae* serovar 61:k:1,5,(7) in Swiss flocks by canton and region in 2017/2018.

Canton and Region	Number of flocks tested (n)	Number of flocks tested positive (y)	Prevalence (%)	95%CI[a]	
Bern	21	9	42.9	21.8	65.9
Fribourg	5	3	60.0	14.7	94.7
Jura	3	0	0.0	0.0	70.7
Neuchâtel	1	1	100	2.5	100
Solothurn	4	2	50.0	6.8	93.2
Region Espace Mittelland	**34**	**15**	**44.1**	**27.2**	**62.1**
Geneva	1	1	100	2.5	100
Vaud	7	4	57.1	18.4	90.1
Valais	13	2	15.4	1.9	45.4
Ticino	6	4	66.7	22.3	95.7
Region lake Geneva & Ticino	**27**	**11**	**40.7**	**22.4**	**61.2**
Aargau	8	4	50.0	15.7	84.3
Baselland	3	1	33.3	0.8	90.6
Zurich	6	1	16.7	0.4	64.1
Region North-West Switzerland & Zurich	**17**	**6**	**35.3**	**14.2**	**61.7**
Appenzell-Innerrhoden	1	1	100	2.5	100
Appenzell-Ausserrhoden	3	1	33.3	0.8	90.6
Glarus	2	2	100	15.8	100
Graubünden	15	12	80.0	51.9	95.7
St. Gallen	14	10	71.4	41.9	91.6
Schaffhausen	1	1	100	2.5	100
Thurgau	6	3	50.0	11.8	88.2
Region Eastern Switzerland	**42**	**30**	**71.4**	**55.4**	**84.3**
Luzern	7	3	42.9	9.9	81.6
Nidwalden	2	1	50.0	1.3	98.7
Obwalden	2	2	100	15.8	100
Schwyz	7	4	57.1	18.4	90.1
Zug	3	1	33.3	0.8	90.6
Region Central Switzerland	**21**	**11**	**52.4**	**29.8**	**74.3**
Total	**141**	**73**	**51.7**	**43.2**	**60.3**

[a] Lower CI and Upper CI indicate 95% Clopper-Pearson confidence intervals

Table 3
Prevalence of *Salmonella enterica* subsp. *diarizonae* serovar 61:k:1,5,(7) in Swiss sheep by age in 2017/2018.

Age category	Number of sheep tested (n)	Number of sheep tested positive (y)	Prevalence (%)	95%CI[a]	
Adult	494	114	23.1	19.4	27.1
Yearling	76	15	19.7	11.5	30.5
Sheep under one year/ lamb	100	11	11.0	5.6	18.8
No information	11	6	54.6	23.4	83.3

[a] Lower CI and Upper CI indicate 95% Clopper-Pearson confidence intervals

(TAZ), chloramphenicol (CHL), ciprofloxacin (CIP), colistin (COL), gentamicin (GEN), meropenem (MERO), nalidixic acid (NAL), sulfamethoxazole (SMX), tetracycline (TET), tigecycline (TGC) and trimethoprim (TMP). In brief, *Salmonella* isolates were regrown from cryopreservation on TSA SB (37 °C ± 1 °C for 24 h ± 3 h aerobically) and colonies were used to achieve an inoculum of approximately 1×10^5 cfu/mL in cation-adjusted Mueller–Hinton broth (CAMHB, Thermo Fisher Scientific (TREK Diagnostic Systems)). Commercially available Sensititre test plates (EUVSEC, Thermo Fisher Scientific (TREK Diagnostic Systems)) were inoculated using an autoinoculator (Thermo Fisher Scientific (TREK Diagnostic Systems)). The plates were incubated at 36 °C ± 1 °C under aerobic atmosphere for 24 h ± 3 h. The reference

Table 4
Prevalence of *Salmonella enterica* subsp. *diarizonae* serovar 61:k:1,5,(7) in Swiss sheep by breed in 2017/2018.

Breed	Number of sheep tested (n)	Number of sheep tested positive (y)	Prevalence (%)	95%CI[a]	
Brown-headed meat sheep	54	17	31.5	19.5	45.6
Bündner Oberland sheep	5	1	20.0	0.5	71.6
Charolais sheep	16	5	31.2	11.0	58.7
Dorper sheep	4	1	25.0	0.6	80.6
Engadiner sheep	24	6	25.0	9.8	46.7
Fox sheep	2	0	0.0	0.0	84.2
German grey heath	17	1	5.9	0.1	28.7
Ile de France sheep	4	1	25.0	0.6	80.6
Jezersko–Solčava sheep	1	0	0.0	0.0	97.5
Lacaune sheep	5	1	20.0	0.5	71.6
Mixed breed	61	19	31.1	19.9	44.3
Nolana sheep	5	0	0.0	0.0	52.2
East Fresian sheep	4	0	0.0	0.0	60.2
Ouaissant sheep	1	0	0.0	0.0	97.5
Scottish blackface sheep	5	0	0.0	0.0	52.2
Black-brown mountain sheep	79	19	24.1	15.1	35.0
Spotted sheep	10	5	50.0	18.7	81.3
Black-headed meat sheep	4	0	0.0	0.0	60.2
Shropshire sheep	5	0	0.0	0.0	52.2
Skudde	5	0	0.0	0.0	52.2
Saaser Mutten sheep	5	0	0.0	0.0	52.2
Valais blacknose sheep	62	0	0.0	0.0	5.8
Spiegelschaf	8	3	37.5	8.5	75.5
Suffolk sheep	6	0	0.0	0.0	45.9
Texel sheep	15	4	26.7	7.8	55.1
Tyrol mountain sheep	4	1	25.0	0.6	80.6
Swiss white alpine sheep	200	47	23.5	17.8	30.0
White-headed meat sheep	5	3	60.0	14.7	94.7
Valais Landrace	1	0	0.0	0.0	97.5
Zackel sheep	1	0	0.0	0.0	97.5
No information	58	11	19.0	9.9	31.4

[a] Lower CI and Upper CI indicate 95% Clopper-Pearson confidence intervals

strain *Escherichia coli* ATTC 25922 was used for quality control and showed MICs within the acceptable range.

2.5. Data analysis

Individual animals were considered either negative or positive for *S.* IIIb 61:k:1,5,(7) according to the results of the laboratory analysis. A flock was defined as either negative or positive as soon as one single animal tested positive for *S.* IIIb 61:k:1,5,(7).

Prevalence and 95% Clopper-Pearson confidence intervals of *S.* IIIb 61:k:1,5,(7) were calculated for the entire Swiss population based on the number of animals and flocks independent of age, canton or breed, as well as separated by canton, breed and age. As some cantons had only a few samples, we additionally merged the cantons into larger regions, namely, "Espace Mittelland" (cantons Bern, Fribourg, Jura, Neuchâtel, Solothurn), "Lake Geneva & Ticino" (Geneva, Vaud, Valais, Ticino), "North-West Switzerland & Zurich" (Aargau, Baselland, Zurich), "Eastern Switzerland" (Appenzell Inner- and Ausserhoden, Glarus, Graubünden, St. Gallen, Schaffhausen, Thurgau) and "Central Switzerland" (Luzern, Nidwalden, Obwalden, Schwyz, Zug).

Eleven samples had missing information for the age of the respective animal and 58 samples had missing breed information. Consequently, these samples were not considered in the calculations separated by age and breed.

Prevalence is provided descriptively, and due to the explorative nature of this study, no inferential statistics were performed. Because sample sizes varied strongly between cantons, breeds and ages, we specifically highlight results with 30 or more samples, as these provide an acceptable reliability of the calculated prevalence for our purposes with an estimated precision of ± 15%.

Because of the previously-mentioned restrictions of the dataset, we investigated only the significance of the differences in the prevalence of *S.* IIIb 61:k:1,5,(7) in flocks between the different regions and age groups. The statistical comparison was based on Kruskal-Wallis rank sum test (library statistics in R). A pairwise comparison using Wilcoxon rank sum test (including a p-value Bendjamini-Hochberg BH correction) was used i) on one hand between Eastern Switzerland and Espace Mittelland; and ii) on the other hand between Eastern Switzerland and Lake Geneva/Ticino. We applied a Kruskal-Wallis rank sum test and the post hoc Wilcoxon rank sum test with correction (BH) on prevalence in flocks between the age categories.

Based on the MIC, isolates were defined as microbiologically susceptible or resistant to the antimicrobial tested according to the epidemiological cut-off values (ECOFFs) for *Salmonella enterica* issued by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (www.eucast.org).

3. Results

3.1. Prevalence of S. IIIb 61:k:1,5,(7) in Swiss sheep

A total of 146 out of 681 nasal swabs from clinically healthy sheep tested positive for *S.* IIIb 61:k:1,5,(7), which corresponds to a prevalence on animal level of 21% (18–25%) (Table 1). In 73 out of 141 flocks tested, at least one sheep tested positive for *S.* IIIb 61:k:1.5,(7), resulting in a minimal prevalence on flock level of 52% (95%CI 43–60%) (Table 2). There was no positive flock, in which all animals tested were positive for *S.* IIIb 61:k:1.5,(7). No other *Salmonella* species were detected.

Salmonella IIIb 61:k:1.5,(7) was detected in nasal swabs of sheep in all cantons except the canton of Jura (Fig. 1), however, only ten animals from three flocks had been sampled there (Table 1, Table 2). Cantons with an evaluable sample size (>=30 animals) showed considerable variation, with a prevalence ranging from 6% (2–15%, Valais) to 39% (28–52%, St. Gallen) (Table 1). Regional prevalence ranged between 14% and 18% for four regions, with the exception of "Eastern Switzerland" with a prevalence of 35%. All cantons constituting the region "Eastern Switzerland" had a prevalence above 30%, except for Thurgau (11%), however, sample sizes per canton in this region varied from 5 to 75 animals per canton. The other regions had wider ranges in the prevalence of the underlying cantons. On the flock level, the regions gave a significant difference overall based on the Kruskal-Wallis rank sum test (p-value of 0.003693). With pairwise comparison using the Wilcoxon rank sum test the region "Eastern Switzerland" showed a significantly higher prevalence (35% (28–42%)) than the Espace Mittelland (adjusted p-value 0.0091) and Lake Geneva/Ticino (adjusted p-

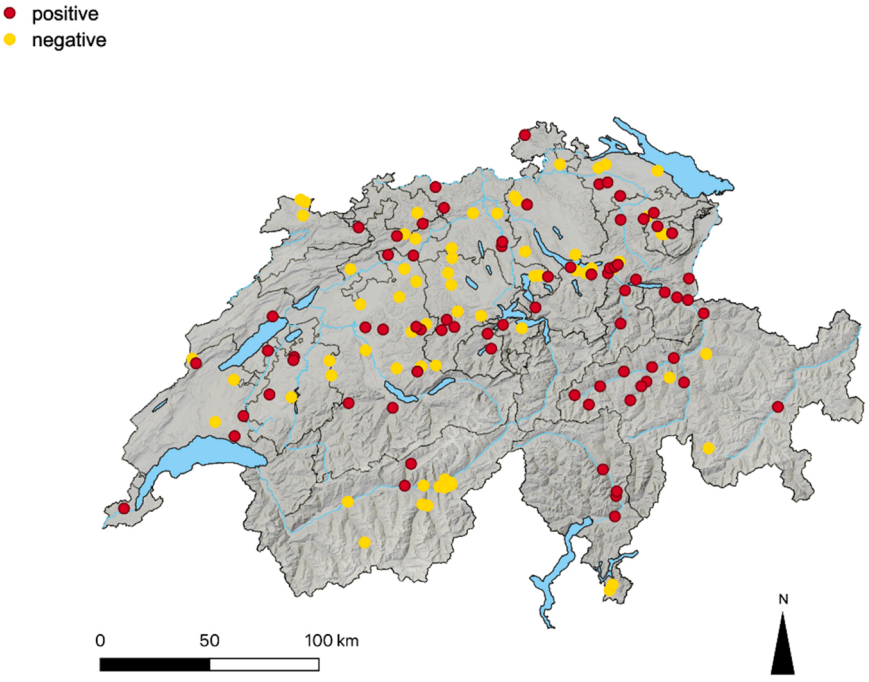

Fig. 1. Swiss sheep flocks tested positive or negative for *Salmonella enterica* subspecies *diarizonae* (IIIb) serovar 61:k:1,5,(7) in 2017/2018.

value 0.0185) regions.

Prevalence increased with age, ranging from 11% (6–19%) in sheep under one year/lambs, to 20% (12–31%) in yearlings, to 23% (19–27%) in adults (Table 3). According to the Kruskal-Wallis rank sum test and the post hoc Wilcoxon rank sum test with correction (BH), adults were significantly (p-value 0.0013) more positive than sheep under one year/lambs.

A total of 29 different breeds were present on the flocks selected for sampling (Table 4). The number of available samples per breed varied substantially. Four breeds, the Brown-Headed Meat sheep (Braunköpfiges Fleischschaf), Black-Brown Mountain sheep (Schwarzbraunes Bergschaf), Valais Blacknose sheep (Walliser Schwarznasenschaf) and the Swiss White Alpine sheep (Weisses Alpenschaf) provided sample sizes of more than 30 animals, with a prevalence of 32% (20–46%), 24% (16–35%), 0% (0–6%) and 24% (18–30%), respectively (Table 4).

3.2. Antimicrobial resistance of S. IIIb 61:k:1,5,(7)

Except for one isolate, all isolates were microbiologically susceptible to all antimicrobials tested (Table 5). One isolate showed microbiological resistance to ampicillin. For colistin, sulfamethoxazole and tigecycline, no ECOFFs were defined. For colistin, the isolates exhibited equally distributed MIC values of 1 mg/L (n = 77) and 2 mg/L (n = 69), which are below or equal to the clinical breakpoint in brackets issued by EUCAST. For tigecycline, all isolates showed MICs below 1 mg/L. For sulfamethoxazole the isolates showed a unimodal MIC distribution with MIC values between < =8 mg/L (n = 13) and 64 mg/L (n = 4).

4. Discussion

The prevalence of *S.* IIIb 61:k:1,5,(7) in nasal swabs from clinically healthy Swiss sheep was 52% at the flock level and 21% at the animal level. Given the low sample size per flock (approximately five sampled animals per flock), the true prevalence at the flock level is likely higher. Assuming a homogeneous prevalence of approximately 20% at the sheep level, the likelihood of sampling five negative animals from a positive flock is approximately 28% (assuming a flock size of 29 sheep). Thus, maintaining the aforementioned assumption, approximately 19 of the 68 flocks without positive samples may have been sampled negatively by chance, resulting in an estimated prevalence on flock level of 65%. This prevalence is very high, especially in view of the fact that the overall prevalence of *Salmonella* spp. in Swiss sheep is negligible. Salmonellosis is a notifiable disease in sheep. There are less than ten cases of *Salmonella* spp. each year (InfoSM, Federal Food Safety and Veterinary Office). This is in line with the finding that we did not detect *Salmonella* serovars other than *S.* IIIb 61:k:1,5,(7) in both, nasal swabs (this and the previous study (Stokar-Regenscheit et al., 2017) and faecal samples (previous study (Stokar-Regenscheit et al., 2017)). Of note, this prevalence is much higher than that of *Salmonella* spp. prevalence in other food producing animals in Switzerland (InfoSM, Federal Food Safety and Veterinary Office).

Several studies on the prevalence of *S.* IIIb 61:k:1,5,(7) in ovine faeces from healthy sheep reported low prevalence on the farm level in northern European countries such as Iceland (0%), Norway (14%) and Sweden (18%). In the latter study, the prevalence in flocks < 30 sheep was much lower (12%) than in flocks with > 30 sheep (40%) (Hjartardóttir et al., 2002; Sandberg et al., 2002; Sörén et al., 2015). In

Table 5
Minimal inhibitory concentration (MIC) distributions of 146 *Salmonella enterica* subspecies *diarizonae* (IIIb) serovar 61:k:1,5,(7) isolates from ovine nasal swabs in 2017/2018.

	MIC[+] (mg/L)																	Microbiological resistance
Antimicrobial agent	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	(%)
Ampicillin (AMP)							132	13		1									0.7
Azithromycin (AZI)										142	4								0
Cefotaxime (FOT)					146														0
Ceftazidime (TAZ)						146													0
Chloramphenicol (CHL)										146									0
Ciprofloxacin (CIP)	137	9																	0
Colistin (COL)							77	69											ND*
Gentamicin (GEN)						146													0
Meropenem (MERO)		146																	0
Nalidixic acid (NAL)									146										0
Sulfamethoxazole (SMX)										13	57	72	4						ND
Tetracycline (TET)								145	1										0
Tigecycline (TGC)					132	14													ND
Trimethoprim (TMP)					143	3													0

[+]MIC: Minimal inhibitory concentration, *ND: Not defined
White areas indicate the range of dilution steps tested for each antimicrobial agent; values above this range signify MIC values higher than the highest concentration tested; values at the lowest concentration tested indicate MIC values equal to or lower than to the lowest concentration tested. Vertical lines indicate the (tentative) epidemiological cut off values (ECOFF) for *Salmonella enterica* issued by the European Committee on Antimicrobial Susceptibility Testing (EUCAST), date 10th January 2022 (www.eucast.org).

+MIC: Minimal inhibitory concentration, *ND: Not defined
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contrast, sheep flocks in Germany (82%) and the US (72%) revealed a much higher flock prevalence of *S*. IIIb 61:k:1,5,(7) on the basis of faecal samples from clinically healthy sheep (Dargatz et al., 2015; Methner and Moog, 2018). In previous studies, the detection proportion of *S*. IIIb 61:k:1,5,(7) in faecal samples resulted in a lower prevalence than in nostrils (Stokar-Regenscheit et al., 2017; Figueras et al., 2020). Therefore, it can be assumed that the prevalence in the abovementioned countries would be even higher if samples from the nose were included. The marked differences in prevalence for *S*. IIIb 61:k:1.5,(7) between countries may be due to different sheep flock sizes, as several studies demonstrated a correlation between prevalence of *S*. IIIb 61:k:1,5,(7) and flock size with higher prevalence in larger flocks (Sörén et al., 2015; Methner and Moog, 2018). The median flock size of the sheep in our study was small (n = 29). Considering the abovementioned findings, the prevalence of *S*. IIIb 61:k:1,5,(7) at the flock level in Switzerland, seems to be much higher than that in Northern Europe but more comparable to that found in Middle Europe or the US, although flock sizes were smaller. Only in Spain was a comparable study to our study conducted on the basis of nasal secretion, which revealed a very high prevalence of *S*. IIIb 61:k:1,5,(7) of 90% on the flock level (Figueras et al., 2020). However, only ten flocks located in the region of Aragon were sampled, therefore lacking representativity.

In our study, the within-flock prevalence of *S*. IIIb 61:k:1,5,(7) (21%) was much lower than the estimated flock prevalence (65%) (Table 1). We showed, that in positive flocks, never all five sheep sampled tested positive for *S*. IIIb 61:k:1,5,(7). This is also in line with the publication of Sandberg et al. (2002), which assumed that transmission from sheep to sheep does not occur easily. *Salmonella* spp. are known to be able to survive for long periods in the environment, which may play a role in maintaining the pathogen within a flock without shedders but carriers. Detailed knowledge on the transmission routes between animals and flocks is missing, and further studies are needed to reveal the factors involved in spreading and environmental contamination of *S*. IIIb 61:k:1,5,(7).

In our study, the prevalence of *S*. IIIb 61:k:1,5,(7) in adults was significantly higher than that in sheep under one year/lambs (Table 3). These results are in line with the findings of our previous study, where only adults but no lambs were found to be positive for *S*. IIIb 61:k:1,5,(7) with nasal swabs (Stokar-Regenscheit et al., 2017). Moreover, other studies showed a positive correlation of age and positivity for *S*. IIIb 61:k:1,5,(7) (Alvseike and Skjerve, 2002; Dargatz et al., 2015). This observation could indicate that colonisation of ovine nasal cavities and subsequent shedding of *S*. IIIb 61:k:1,5,(7) may take several months for unknown reasons. For instance, lambs may be protected by maternal antibodies and/or elder sheep get colonized only in an immunosuppressed status (e. g. pregnancy or stress) and/or insufficient hygiene management status on flock.

Flocks positive for *S*. IIIb 61:k:1,5,(7) were detected in almost all cantons of Switzerland (Table 1). The prevalence of *S*. IIIb 61:k:1,5,(7) differed markedly between the cantons, but for some cantons the sample size was very small, leading to a potential overestimation of prevalence at both, the flock and animal levels. The region of Eastern Switzerland showed a significantly higher prevalence at the flock level. Within this region, the canton Graubünden was included, which has a high density of alps. In general, in mountainous cantons there were more sheep flocks to be sampled than in rather flat cantons, without the flock sizes being larger.

Concerning the breeds, we could show that not only Texel sheep are susceptible to nasal colonisation with *S*. IIIb 61:k:1,5,(7), but several breeds were affected (Table 4). Notably, the Valais black-nosed sheep (Schwarznasenschaf) did not show one single positive animal (Table 4), although it was tested just as often (n = 62) as the Black-brown Mountain sheep (n = 82) and the Brown-headed sheep (n = 54). This might indicate a reduced susceptibility to colonisation with *S*. IIIb 61:k:1,5,(7).

No microbiological resistance was found in 146 Swiss *S*. IIIb 61:k:1,5,(7) isolates. This is consistent with results from a previous Swiss study with comparable methods and the findings of the published genome sequence of a German *S*. IIIb 61:k:1,5,(7) isolate from 2019 (Schnydrig et al., 2018; Uelze et al., 2019). In contrast, high resistance to sulfamethoxazole was found in two older studies from Germany and Switzerland (Bonke et al., 2012; Methner and Moog, 2018).

In summary, our study revealed a high nasal colonisation proportion with *S*. IIIb 61:k:1,5,(7) in clinically healthy Swiss sheep flocks, which is accompanied by a lower prevalence at the animal level. Concerning the zoonotic significance on public health, European countries such as Sweden and Germany considered this to be negligible, as the risk of transmission via sheep meat is very low and human cases with *S*. IIIb 61:k:1,5,(7) occur only sporadically (Sörén et al., 2015, Methner and Moog, 2018). For this reason, in Sweden *S*. IIIb 61:k:1,5,(7) was excluded from the national *Salmonella* control program. To the best of the authors' knowledge, *S*. IIIb 61:k:1,5,(7) has thus far not been detected in meat, meat products or cheese from sheep in Switzerland.

It remains unclear, whether and how the high prevalence of *S*. IIIb 61:(k)k:1,5,(7) in nasal cavities impacts the upper respiratory tract of Swiss sheep. Figueras et al. (2020) showed that the prevalence of *S*. IIIb 61:k:1,5,(7) in flocks with previous cases of CPR had a higher likelihood of harbouring a sheep that tested positive compared to flocks without a previous history of CRP. Worth mentioning in this context is the fact that, the majority of antimicrobial treatment in Swiss sheep is due to respiratory tract disorders.

Although several studies have tried to elucidate the factors that trigger or are involved in the transformation from colonisation to infection of *S*. IIIb 61:k:1,5,(7) in sheep, no clear picture can currently be taken. Experimental infection of ewes with *S*. IIIb 61:k:1,5,(7) did not result in abortion or other clinical signs (Hannam et al., 1986). Oral infection of lambs did not provoke clinical signs consistently in all infected lambs, but all of them shed *S*. IIIb 61:k:1,5,(7) with faeces and pathological findings confirming intestinal inflammation (Lacasta et al., 2017). Intranasal infection of lambs did not induce clinical disorders or pathological findings, but the pathogen was detectable in faeces and nasal secretions. These results may suggest that other factors are needed to provoke clinical inflammation in the upper respiratory tract of sheep (Lacasta et al., 2017). Comparative analysis of the genome of *S*. IIIb 61:k:1,5,(7) isolates revealed indicators for host adaptation, such as the number of pseudogenes and genomic rearrangements and with sequence type (ST) 432, a specific sheep adapted lineage could be identified (Uelze et al., 2019, 2021). Comparative studies on the genome of *S*. IIIb 61:k:1,5,(7) isolates from both, clinically healthy and diseased sheep, may shed light on factors expressed by the pathogen involved in infection and virulence.

5. Conclusion

The estimated flock prevalence of *S*. IIIb 61:k:1,5,(7) in clinically healthy Swiss sheep of 65% indicates that this *Salmonella* serovar might be endemic in Swiss sheep flocks. The importance of *S*. IIIb 61:k:1,5,(7) on public health seems to be negligible, and the level of antimicrobial resistance in *S*. IIIb 61:k:1,5,(7) was very low (0.7% for ampicillin). In contrast, further research should focus on the pathogenic impact of *S*. IIIb 61:k:1,5,(7) on the health status of sheep, especially of disease of the upper respiratory tract.

Conflict of Interest Statement

The authors declare no conflicts of interest.

Acknowledgements

The author would like to thank the persons in the field involved in sampling for their cooperation, especially Gaia Moore-Jones. We would like to thank the participating farmers and animal owners for their cooperation and helpfulness, the Federal Food Safety and Veterinary

Office (FSVO) for the financial support (1.17.05) of the sampling and the Federal Office for Agriculture (FOAG) for providing access to the contact information of participants. Moreover, many thanks to the stuff of the Institute of Veterinary Bacteriology, University of Bern, especially Susanne Rickli and Andreas Thomann for their help in performing the bacteriological analyses. This study was financed by the Institute of Veterinary Bacteriology, University of Bern.

References

Alvseike, O., Skjerve, E., 2002. Prevalence of a *Salmonella* subspecies *diarizonae* in Norwegian sheep herds. Prev. Vet. Med. 52, 277–285. https://doi.org/10.1016/s0167-5877(01)00252-5.

Ardüser, F., Moore-Jones, G., Gobeli Brawand, S., Dürr, S., Steiner, A., Ryser-Degiorgis, M.P., Zanolari, P., 2020. *Dichelobacter nodosus* in sheep, cattle, goats and South American camelids in Switzerland-Assessing prevalence in potential hosts in order to design targeted disease control measures. Prev. Vet. Med. 178, 104688 https://doi.org/10.1016/j.prevetmed.2019.05.001.

Bonke, R., Wacheck, S., Bumann, C., Thum, C., Stüber, E., König, M., Stephan, R., Fredriksson-Ahomaa, M., 2012. High prevalence of *Salmonella enterica* subsp. *diarizonae* in tonsils of sheep at slaughter. Food Res. Int. 45, 880–884 https://doi.org/0.1016/j.foodres.2011.01.050.

Chatzopoulos, D.C., Sarrou, S., Vasileiou, N.G.C., Ioannidi, K.S., Peteinaki, E., Valiakos, G., Tsokana, C.N., Papadopoulos, E., Spyrou, V., Mavrogianni, V.S., Giannakopoulos, A., Sbiraki, A., Lacasta, D., Bueso, J.P., Athanasiou, L.V., Billinis, C., Fthenakis, G.C., 2016. Dissemination of intestinal pathogens between lambs and puppies in sheep farms. Small Rumin. Res.: J. Int. Goat Assoc. 141, 5–10. https://doi.org/10.1016/j.smallrumres.2016.06.006.

CLSI, 2018. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals, 5th ed. CLSI standard VET01. Wayne, PA: Clinical and Laboratory Standards Institute.

Dargatz, D.A., Marshall, K.L., Fedorka-Cray, P.J., Erdman, M.M., Kopral, C.A., 2015. *Salmonella* prevalence and antimicrobial susceptibility from the national animal health monitoring system sheep 2011 study. Foodborne Pathog. Dis. 12, 953–957. https://doi.org/10.1089/fpd.2015.2016.

Davies, R.H., Evans, S.J., Preece, B.E., Chappell, S., Kidd, S., Jones, Y.E., 2001. Increase in *Salmonella enterica* subspecies *diarizonae* serovar 61:k:1,5,(7) in sheep. Vet. Rec. 149, 555–557. https://doi.org/10.1136/vr.149.18.555.

Ferreras Mdel, C., Muñioz, M., Pérez, V., Benavides, J., García-Pariente, C., Fuertes, M., Adúriz, G., García-Marín, J.F., 2007. Unilateral orchitis and epididymitis caused by *Salmonella enterica* subspecies *diarizonae* infection in a ram. J. Vet. Diagn. Investig.: Off. Publ. Am. Assoc. Vet. Lab. Diagn., Inc. 19, 194–197. https://doi.org/10.1177/104063870701900211.

Figueras, L., Ferrer, L.M., González, J.M., Bueso, J.P., Ramos, J.J., Rubira, I., Burian, E., Lacasta, D., 2020. Prevalence of *Salmonella enterica* subsp. *diarizonae* serotype 61:k:1:5:(7) in nasal secretions and stool of sheep flocks with and without cases of chronic proliferative rhinitis. Vet. Microbiol. 247, 108767 https://doi.org/10.1016/j.vetmic.2020.108767.

Gupta, S.K., McMillan, E.A., Jackson, C.R., Desai, P.T., Porwollik, S., McClelland, M., Hiott, L.M., Humayoun, S.B., Barrett, J.B., Frye, J.G., 2016. Draft Genome Sequence of *Salmonella enterica* subsp. *diarizonae* serovar 61:k:1,5,(7) Strain CRJJGF_00165 (Phylum *Gammaproteobacteria*). e01322-01316 Genome Announc. 4. https://doi.org/10.1128/genomeA.01322-16.

Hannam, D.A., Wray, C., Harbourne, J.F., 1986. Experimental *Salmonella arizonae* infection of sheep. Br. Vet. J. 142, 458–466. https://doi.org/10.1016/0007-1935(86)90048-5.

Hjartardóttir, S., Gunnarsson, E., Sigvaldadóttir, J., 2002. *Salmonella* in sheep in Iceland. Acta Vet. Scand. 43, 43–48. https://doi.org/10.1186/1751-0147-43-43.

Hyeon, J.Y., Helal, Z.H., Polkowski, R., Heishima, M., Kim, J., Lee, D.H., Risatti, G.R., 2021. Genetic features of *Salmonella enterica* subspecies *diarizonae* serovar 61:k:1,5 isolated from abortion cases in sheep, United States, 2020. Res Vet. Sci. 138, 125–136. https://doi.org/10.1016/j.rvsc.2021.06.007.

Lacasta, D., Ferrer, L.M., Ramos, J.J., Bueso, J.P., Borobia, M., Ruiz de Arcaute, M., Figueras, L., González-Sainz, J.M., De Las Heras, M., 2012. Chronic proliferative rhinitis associated with *Salmonella enterica* subspecies *diarizonae* serovar 61:k:1, 5, (7) in sheep in Spain. J. Comp. Pathol. 147, 406–409. https://doi.org/10.1016/j.jcpa.2012.03.004.

Lacasta, D., Figueras, L., Bueso, J.P., De las Heras, M., Ramos, J.J., Ferrer, L.M., González, J.M., Ruíz de Arcaute, M., Ortín, A., Marteles, D., Navarro, T., Fernández, A., 2017. Experimental infection with *Salmonella enterica* subsp. *diarizonae* serotype 61:k:1,5,(7) in sheep: Study of cell mediated immune response. Small Rumin. Res. 149, 28–33.

Meehan, J.T., Brogden, K.A., Courtney, C., Cutlip, R.C., Lehmkuhl, H.D., 1992. Chronic proliferative rhinitis associated with *Salmonella arizonae* in sheep. Vet. Pathol. 29, 556–559. https://doi.org/10.1177/030098589202900616.

Methner, U., Moog, U., 2018. Occurrence and characterisation of *Salmonella enterica* subspecies *diarizonae* serovar 61: k: 1, 5, (7) in sheep in the federal state of Thuringia, Germany. BMC Vet. Res. 14, 401. https://doi.org/10.1186/s12917-018-1741-4.

Sandberg, M., Alvseike, O., Skjerve, E., 2002. The prevalence and dynamics of *Salmonella enterica* IIIb 61:k:1,5,(7) in sheep flocks in Norway. Prev. Vet. Med. 52, 267–275. https://doi.org/10.1016/s0167-5877(01)00250-1.

Schnydrig, P., Overesch, G., Regli, W., Bee, A., Rodriguez-Campos, S., 2018. *Salmonella enterica* subspecies *diarizonae* serovar 61:(k):1,5,(7) as cause of caprine abortion. Small Rumin. Res. 166, 78–82. https://doi.org/10.1016/j.smallrumres.2018.07.022.

Sörén, K., Lindblad, M., Jernberg, C., Eriksson, E., Melin, L., Wahlström, H., Lundh, M., 2015. Changes in the risk management of *Salmonella enterica* subspecies *diarizonae* serovar 61:(k):1, 5, (7) in Swedish sheep herds and sheep meat due to the results of a prevalence study 2012. Acta Vet. Scand. 57 (6) https://doi.org/10.1186/s13028-015-0096-0.

Stokar-Regenscheit, N., Overesch, G., Giezendanner, R., Roos, S., Gurtner, C., 2017. *Salmonella enterica* subsp. *diarizonae* serotype 61:k:1,5,(7) associated with chronic proliferative rhinitis and high nasal colonisation proportions in a flock of Texel sheep in Switzerland. Prev. Vet. Med. 145, 78–82. https://doi.org/10.1016/j.prevetmed.2017.07.003.

Uelze, L., Borowiak, M., Deneke, C., Fischer, J., Flieger, A., Simon, S., Szabó, I., Tausch, S.H., Malorny, B., 2021. Comparative genomics of *Salmonella enterica* subsp. *diarizonae* serovar 61:k:1,5,(7) reveals lineage-specific host adaptation of ST432. Microb. Genom. 7 https://doi.org/10.1099/mgen.0.000604.

Uelze, L., Borowiak, M., Deneke, C., Jacobs, C., Szabó, I., Tausch, S.H., Malorny, B., 2019. First complete genome sequence and comparative analysis of *Salmonella enterica* subsp. *diarizonae* serovar 61:k:1,5,(7) indicates host adaptation traits to sheep. Gut Pathog. 11, 48. https://doi.org/10.1186/s13099-019-0330-9 eCollection 2019.

Uelze, L., Borowiak, M., Flieger, A., Simon, S., Tausch, S.H., Malorny, B., 2020. Complete Genome Sequence of Salmonella enterica subsp. diarizonae Serovar 61:k:1,5,(7) Strain 14-SA00836-0, Isolated from Human Urine. Microbiol Resour Announc 9 (36), 00683–00720. https://doi.org/10.1128/MRA.00683-20.

Zweifel, C., Zychowska, M.A., Stephan, R., 2004. Prevalence and characteristics of Shiga toxin-producing *Escherichia coli, Salmonella* spp. and *Campylobacter* spp. isolated from slaughtered sheep in Switzerland. Int. J. Food Microbiol. 92, 45–53. https://doi.org/10.1016/j.ijfoodmicro.2003.07.005.